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Some Cultural and Cytological Characteristics of Human Tumors *in Vitro**†

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INTRODUCTION

The present study was undertaken to determine both the general growth patterns and some of the cytological details of frequently occurring human tumors. For this purpose the roller tube method, which provides good nutritional conditions, and the hanging drop method, which, less favorable nutritionally, provides optimal conditions for cytological investigation, were used.

The cultural behavior of human tumors grown in roller tube cultures has been observed by Gey and Gey (8) and Coman (4, 5). Other investigators, among them Kredel (11); Buckley (1); Russell and Bland (16); Zakrzewski and Kraszewski (17); Pinkus (15); Höfer (10); Grand, Chambers, and Cameron (9); and Murray and Bradley (14), have studied the cultural characteristics of human tumors in hanging drop slides or Carrel flasks and incidentally have included observations on the cytology of their cells. It therefore seemed worth while to study primarily the cytology of the stroma and parenchyma, including the shapes of the cells and their nuclei, the condition of the cytoplasm and nucleoplasm, the form and distribution of mitochondria and neutral red granules, the shapes and number of nucleoli, and the process of division whenever possible; and, secondarily, to note any individual peculiarities in the cultural behavior of these neoplasms.

METHOD

Twenty human tumors, obtained from the operating room, were cultured in a roller tube (Coman and Stabler, 3) and subcultured in hanging drop slides. Specimens were grown in the roller tubes in a chicken plasma and chick embryo extract clot and a fluid medium of 10 drops of physiological saline (8) and 15 drops of fetal cord serum according to the method described by Coman (4). The subcultures on slides were grown in a medium of 5 parts embryo extract, 3 parts plasma, 3 parts physiological saline (8) and 2 parts fetal cord serum. Most of the cytological data were obtained when the preparations were 1 to 4 days old. When cultures were studied beyond this point the medium was renewed.

Studies of mitochondria were made on hanging drop preparations placed in a 1:50,000 Janus Green B solution for 15 minutes, and studies of neutral red granules on hanging drop cultures stained in 1:2,500 neutral red solution for 15 minutes. Preparations were discarded 1 hour after they had been treated with one or both of these dyes. Some of the vitally stained and some unstained hanging drop cultures were fixed in Bouin's solution and subsequently stained with iron-alum hematoxylin and light green. These were later studied and compared with observations on living cells.

Nucleolar counts in epithelial cells and in fibroblasts were made on a total of 10 hanging drop preparations. Since there were few epithelial outgrowths in all explants of these preparations, cells in every epithelial projection of every explant of a culture were investigated; the number in the outgrowths was small enough so that there was no possibility of counting the same cells twice. However, in fibroblast outgrowths that were more prolific, fields were mapped on the upper, middle, and lower portions of only 1 explant of a culture, and the nucleoli of the cells at only 1 focus of the microscope were counted.

RESULTS

Of the 20 tumors cultured, 3 carcinomas of the breast and 1 adenoma of the thyroid have been selected for presentation. The malignant tumors are discussed in one group, the benign tumor of the thyroid separately.

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Adenoma of the Thyroid

Cultural characteristics.—Within 24 hours after the roller tube cultures had been set up, tufts of fibroblasts projected from the explants and increased in number during the 55 days the cultures were grown until finally they formed solid sheets of cells.

Epithelial cords and tongues projected into the plasma clot by the second day. These cultures were notable in that their first epithelial outgrowths were cords of cells that bent and twisted around and, within 2 days after cultures had been set up, formed acinar structures. Such organized groups of epithelial cells have been previously reported by Cameron and Chambers (2) in a carcinoma of the breast and by Coman (4) in a benign tumor of the breast. Morphologically, the acini found in the cultures of the thyroid adenoma resembled those described by Coman more closely than those described by Cameron and Chambers. They differed, however, in that they appeared after 2 days of culture, whereas in Coman's cultures they were found only after a month *in vitro*. Coman does not report their fate; Cameron and Chambers state that the acinar organizations in their cultures later resolved into epithelial sheets. Those here reported similarly resolved into sheets of epithelium, which grew luxuriantly during the life of the cultures.

Cytological characteristics.—Whether in sheets, cords, or tongues the epithelial cells were for the most part polygonal in shape, though variations of this form were observed on the edge of an outgrowth; such cells were often slightly fusiform. The epithelial nuclei were oval in living cells, and round in fixed and stained hanging drop preparations. Within the nuclei, 2 nucleoli of various sizes were seen; although their shape varied in different cells, they were most often round with an irregular periphery.

The cytoplasm was homogeneous in appearance with the exception of a few granular areas, which either bordered on or were localized at one of two ends of the oval nuclei. Refractile fat globules followed the same pattern of distribution as the granulations. Small clusters of fat surrounded the granular areas at the tips of the nuclei or formed a perinuclear ring between the periphery of the nuclei and cell membrane. Occasionally a row of 2 to 4 globules was found in the peripheral regions of the cells.

Similarly neutral red granules were clumped at either or both ends of the nuclei. Cells varied in the size and quantity of granules contained; in some they were scattered throughout, with the greatest concentration in the region of the nucleus. Perinuclear distribution of mitochondria was also evident in these epithelial cells. Rod-like, filamentous, and spherical mitochondria were seen, the latter most frequently.

No complete data were gathered on the division cycles of this tumor, although cells in mitosis were often found in fixed and stained preparations. Observations were made on an epithelial cell of the adenoma in telophase, at which time the cell was a long oval shape, with its spindle drawn into a long cylinder, at each end of which chromosome rods were visible. Granules and fat globules were aligned on the long axis of the cell between the margin of the spindle and the periphery. Twelve minutes after the observation was begun the central portion of the spindle had thinned, the chromosomes had clumped at either end, and the cytoplasm had started to constrict. As soon as the connection between the divided chromosomes had broken (8 minutes later), granules and fat, previously aligned along the margin of the cell, moved to the center, through which the line of cytoplasmic cleavage ran. The chromosomes lost their identity, and the outlines of the daughter nuclei became irregular and difficult to see. The granules and fat, divided between the two cells, were scattered. Four minutes later, the daughter nuclei started to reorganize and the fat became localized around the periphery of the cell. The time required from the beginning of telophase to the end of cytoplasmic division was 30 minutes. These cells were not observed again until 40 minutes later, when they had the appearance of typical epithelial cells with clearly defined nuclei and faint nucleoli.

The course of the final stages of division of this cell corresponded to that of similar stages in the division of normal animal cells (12) and the epithelial cells of a human squamous cell carcinoma (10).

The stroma cells of the adenoma were usually bipolar, spindle-shaped cells, although broad tripolar fibroblasts with 1 process at the proximal end and 2 at the distal end were also seen in the outgrowths of this tumor. The oval nuclei usually contained 2 nucleoli, which were bent, twisted, and dumbbell-shaped or triangular in surface view. The nucleoplasm was finely granular and appeared much denser than the smooth, homogeneous cytoplasm. The only granules in the cytoplasm were localized at the poles of the nuclei.

The granular areas contained small accumulations of fat globules. In tripolar cells, 2 small clusters of fat appeared peripherally and on opposite sides of the cell body in the region where the 2 cell processes extended. In all fibroblasts an occasional row of 2 to 6 fat globules, resembling a string of beads, appeared in the cell processes parallel to the long axis of the cells. Filamentous mitochondria, the predominant form in these cells, were located in the cell extensions, although filaments, rods, and spherules were also grouped around the nuclei. Unlike mitochondria, neutral red granules were seldom seen in the outer

portions of the cell processes. Most neutral red granules accumulated in the granular parts of the cytoplasm.

Carcinomas of the Breast

Cultural characteristics.—In 2 of the 3 carcinomas, fibroblastic preceded epithelial outgrowth, and appeared on the first and second days; the epithelial cells were seen on the third day. In the third tumor, both components grew out on the fourth day. The patterns of growth were typical of those previously reported. Fibroblasts grew out radially and increased in number during the period of cultivation, so that the explants were eventually surrounded by a halo of densely packed cells. Mingled with these were cords and tongues of epithelium, which grew as sheets of flat polygonal cells.

Two of the breast tumors formed whorls of epithelial cells (Fig. 1) at the peripheries of the zones of outgrowth that often separated from the main outgrowth (Fig. 2), and subsequently became loci of new epithelial colonies from which wide polygonal cells proliferated. In appearance the whorls resembled the pearls described by Cameron and Chambers (2) and by Coman (4) in cultures of squamous cell carcinomas; in behavior they resembled the groups of epithelial cells in a mouse carcinoma described by Fischer, Fischer, and Hollmann (7), in that both migrated from the solid areas of outgrowth to points on the margins of the clots.

It seems probable in the light of the work of Coman (6) that the whorls are a result of decreased cohesiveness in malignant as compared with normal cells. Coman found that it required significantly less force to pull apart two malignant cells than to separate two normal cells from the corresponding normal tissue. The detachment of these clusters is interesting because metastasis is one of the distinguishing characteristics of most malignant tumors *in vivo.* The separation of the whorls *in vitro,* and the subsequent formation of loci of further growth from them, suggest that the behavior of carcinoma cells *in vitro* parallels their behavior *in vivo.*

Cytological characteristics.—Epithelial cells in cultures of the 3 carcinomas of the breast were polygonal, with one centrally placed, broad oval nucleus; possibly as a result of liquefaction of the clot or of the contact of neighboring cells, some were fusiform in shape. The cell membrane, with the exception of cells at the margins of the sheets, was not always visible, although the general limits of each cell were discernible by differences in opacity at the center and along the periphery.

Almost all carcinoma cells contained finely granular, dense cytoplasm, which extended to the periphery exclusive of the ectoplasmic layer and gave them a stippled appearance. Very infrequently carcinoma cells had localized granular areas in otherwise homogeneous cytoplasm. The nuclei of all epithelial cells of these tumors were filled with an even denser, more coarsely granular, heterogeneous nucleoplasm separated from the cytoplasm by distinctly visible nuclear membranes. The condition of the cytoplasm and nucleoplasm of these malignant tumors is in contrast to the condition of the cytoplasm and nucleoplasm of the adenoma of the thyroid, described above, in which granulations were localized in the cytoplasm and fine and evenly dispersed in the nucleoplasm. These observations suggest that diffusely granular, dense cytoplasm and coarsely granular, dense nucleoplasm may be a condition of malignancy in human tumors, as Lewis (13) has suggested in animal tumor cultures.

Counts of the number of nucleoli in malignant epithelial cells showed a range of 1 to 4 per nucleus, with 1 and 2 the most frequent number, 3 and 4 rare. There were obvious size differences in nucleoli in the same nucleus and in nuclei in different cells. Nucleoli were usually round, with smooth contours and occasionally with small indentations.

Very few fat droplets were seen in these cells; when present, they were scattered irregularly throughout the cytoplasm or concentrated perinuclearly. Spherical mitochondria also accumulated around the nuclei. The rod-like and filamentous forms, which appeared less frequently in epithelial cells, were situated at the periphery rather than in the central region. The peripheral distribution of neutral red granules predominated when epithelial cells were unusually broad, but more often they banded the nucleus and extended into the region where there was the greatest amount of cytoplasm (Fig. 3).

The stroma in these cultures was composed of long, thin, bipolar spindle-shaped cells, both ends of which terminated in a long cell process. Their long, oval nuclei, frequently found in the broadest part of the fibroblasts whether this region was in the central area or near either end, was composed of dense but uniformly granular nucleoplasm.

Some nucleoli also seemed granular, as if formed by the accumulation of many small nucleolar fragments. These often contained vacuoles. Others were smooth and nonvacuolated. All were irregularly shaped. Of a total of 476 cells counted, 174 contained 1 nucleolus, 239 contained 2, and 63 contained 3 or more.

If the nuclei were peripheral instead of central, granules accumulated at their sides; otherwise they clustered at one or both poles of the nuclei. The rest of the cell, except for areas in which fat, mitochondria, and neutral red granules were suspended, was nongranular and smooth.

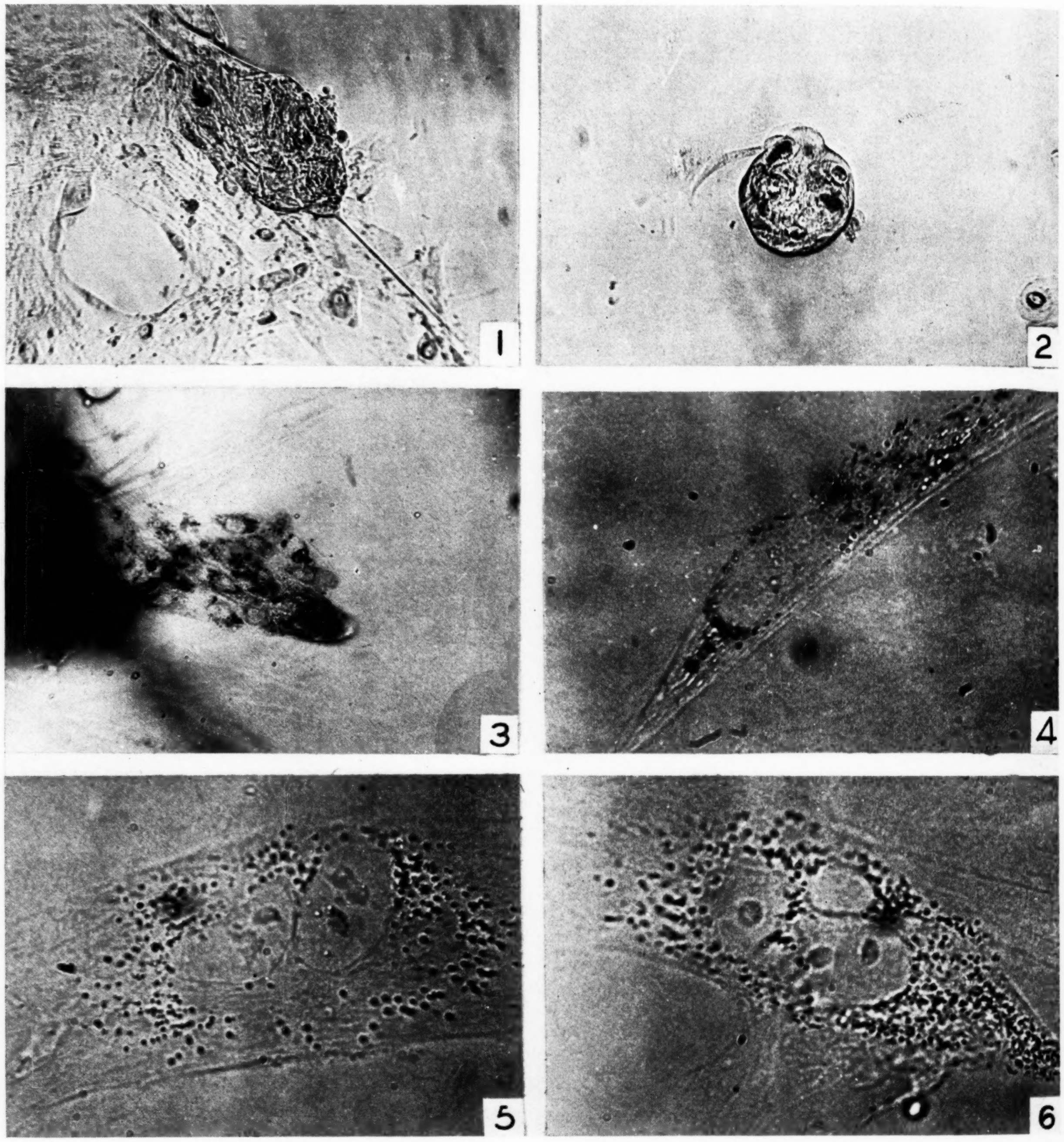

FIG. 1.—Whorls of epithelial cells on margin of zone of outgrowth. Fifteen-day culture of carcinoma of breast. Mag. × 100.

FIG. 2.—Detached whorl showing initial stage of new proliferation. Fifteen-day culture of carcinoma of breast. Mag. × 100.

FIG. 3.—Epithelial tongue showing varying amounts of neutral red granules in perinuclear distribution and scattered in the cytoplasm. Four-day hanging drop preparation of carcinoma of breast. 1:2,500 neutral red. Mag. × 200.

FIG. 4.—Bipolar fibroblast with neutral red granules localized at end of oval nucleus and dispersed in cytoplasm at opposite pole. Highly refractile fat globules. Two-day hanging drop culture of carcinoma of breast. 1:2,500 neutral red. Mag. × 920.

FIG. 5.—Binucleate fibroblast with 2 irregular vacuolated nucleoli in each nucleus. Nucleoplasm homogeneous and nuclear membrane visible. Six-day hanging drop preparation of carcinoma of breast. Mag. × 920.

FIG. 6.—Trinucleate fibroblast. Nuclear components of varying sizes; vacuolated nucleoli with homogeneous contours. Three-day hanging drop preparation of carcinoma of breast. Mag. × 920.

Fat globules were most often scattered in the granular localizations or lined up in rows of several at the ends of the cell processes. Mitochondria were similarly situated, the rods and filaments the most common forms. When the cells were stained with neutral red the various sized granules in which the dye accumulated capped or flanked the nuclei and extended into the processes, except for the outermost tips of the cells (Fig. 4).

Multinucleate fibroblasts and epithelial cells varied in the shape and number of their nuclear components. In an epithelial cell there were 2 round nuclei, separated by a granular area in which mitochondria and neutral red granules accumulated. Other cells had a kidney-shaped and an oval nucleus, two oval nuclei (Fig. 5), or 3 ovoid nuclei of different sizes (Fig. 6). With the exception of one part of this trinucleate cell, which had no nucleoli, nuclear components in multinucleate cells had from 1 to 4 nucleoli each. Nucleoli were round or irregularly shaped, as shown in Figs. 5 and 6 respectively. In both of these, typical of all, nucleoli were heterogeneous in appearance and contained small vacuoles.

A dividing carcinoma cell from one of the cultures containing multinucleate cells was studied for 23 minutes. It was first noticed in anaphase, during which time clear blebs of cytoplasm started to protrude from the cell surface. When it was observed 2 hours after telophase, the cytoplasm had still failed to divide completely. These observations suggest that the formation of binucleate cells resulted from a failure of the cytoplasm to cleave during division.

SUMMARY AND CONCLUSIONS

A study of 3 carcinomas of the breast and 1 adenoma of the thyroid, grown in roller tubes and studied cytologically in hanging drop cultures, is reported.

Cells in outgrowths of the cultures of 2 carcinomas of the breast formed whorls of epithelial cells that subsequently separated from the zones of outgrowth and formed loci of new colonies. It is suggested that the behavior of these whorls *in vitro* parallels the metastatic behavior of malignant epithelial cells *in vivo*.

Spherical mitochondria predominated in epithelial cells, rods and filaments in fibroblasts.

The neutral red granules in both parenchyma and stroma cells were usually in groups about the nucleus, but a few clusters were scattered at the periphery.

Diffuse granularity characterized most malignant epithelial cells, while a homogeneous cytoplasm with localized granular areas was more often present in the epithelial cells of the benign tumor and in the fibroblasts of both benign and maligant tumors.

ACKNOWLEDGMENT

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Potassium and Calcium Content of Gastric Carcinoma*

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It has been demonstrated that with few exceptions the potassium content of malignant neoplasms is increased, whereas the calcium content is decreased, relative to nonmalignant mature tissues (3). An elevated K/Ca ratio has been regarded as characteristic of malignant neoplasms. In 1932 Epstein (2) observed that the potassium content in a variety of human neoplasms was higher than that in the tissues from which these growths developed; included in the series were 2 gastric carcinomas. In the studies recorded below gastric carcinomas were analyzed for potassium and calcium, and the results compared with similar analyses of the grossly uninvolved mucosa both adjacent to the growths and as far away from them as it was possible to obtain samples.

Surgical specimens of stomachs were obtained as quickly as possible after excision and washed in running water. Strips of mucosa were then separated from the muscularis and submucosa and placed in a weighing bottle, and nonnecrotic tumor tissue was sliced and placed in another weighing bottle. These specimens were weighed immediately whenever possible, or placed in the icebox until weighing time. They were also wiped with gauze while in the weighing bottles for removal of blood clots, mucus, etc., and were then minced rapidly with scissors. About 10 gm. of minced tissue were weighed out into a platinum crucible for the Ca determinations, and 1 to 2 gm. into a small silica crucible for K determinations. Tissues weighing 0.2 to 0.5 gm. were weighed in duplicate on small watch glasses and transferred quantitatively, with rinsing, to a micro-Kjeldahl flask for nitrogen determinations. The N digestion was carried out immediately; the other tissues were placed in a 110° C. oven for 48 hours. After desiccation for 1 hour they were reweighed, and the percentage of H_2O was calculated from the difference in weights. Tissues in the crucibles were then covered with ethyl ether and placed in a desiccator for 24 hours; at the end of this period the ether was removed with a micropipette and they were again covered with ethyl ether; this was repeated 3 times with ether and 3 times with petroleum ether, the procedure thus taking 6 days. After drying and desiccation they were reweighed, and the percentage of fat was calculated from differences in these weights. The tissues for Ca determinations were then placed in a muffle furnace at 600° C. for at least 24 hours, or until ashing was complete; 1 drop of HNO_3 was added to complete the ashing if necessary. The tissues for K determinations were charred at 110° C. after the addition of 0.5 to 1.0 cc. of 4 *N* H_2SO_4; after 24 hours' charring they were placed in a muffle furnace at a temperature of 400° to 500° C. until completely ashed. The residue was dissolved in dilute HCl and transferred quantitatively to a 10 cc. volumetric flask; repeated washings with hot H_2O were used to recover all the material from the crucibles. The flasks were allowed to cool, then made up to volume, centrifuged, and the clear solutions used for Ca and K determinations.

Nitrogen determinations were made with the micro-Kjeldahl method by converting N into an ammonium salt and analyzing by the usual volumetric procedure.

K determinations were made in triplicate with the method of Shohl and Bennett, as modified by Dr. Lillian Eichelberger (1). Calcium determinations were carried out in duplicate on 4 cc. aliquots of the acid solution. The Kramer-Tisdall method was followed with modifications for tissue work, as suggested by Dr. Eichelberger; *i. e.*, pH was carefully adjusted to from 4.2 to 4.4 with dilute ammonia, 1 drop of bromcresol green being used as indicator. The Ca was then precipitated as oxalate; after centrifugation the precipitate was washed and dissolved in H_2SO_4 for titration with $KMNO_4$.

The results in 14 specimens of carcinoma of the stomach are summarized in Table I. The average water content of neoplastic and nonneoplastic gastric mucosa was found to be practically identical. The calcium content of the carcinomas was relatively lower than that of the mucosa, and the inverse was true of the potassium content. However, there were a few exceptions: in Cases 7 and 11 the calcium content of carcinoma and mucosa was approximately the same, and in Case 4 the potassium content was lower in the neoplasm than in the mucosa. The total nitrogen in the neoplasms was slightly higher than in the uninvolved mucosa.

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Results of analyses of mucosa from noncancerous control stomachs are shown in Table II. The calcium content of mucosa from noncancerous stomachs was lower than of uninvolved mucosa from cancerous stomachs, and about equal to the calcium content of gastric carcinoma. The potassium content was of the same order as that of the uninvolved mucosa.

In 2 instances the gastric neoplasms proved to be lymphosarcomas. The calcium content in one was 5.1 mgm. per cent for the mucosa and 2.0 mgm. per cent for the neoplastic tissue; in these 2 cases the potassium content of the mucosa was 168 and 169 mgm. per cent respectively, whereas in the neoplasms it was 358 and 229 mgm. per cent. These values correspond to those observed for carcinomas.

In one instance 3 neoplasms were present in the lower stomach; *viz:* infiltrating scirrhous carcinoma just proximal to the pylorus, a benign papilloma proximal to this, and an ulcerating adenocarcinoma proximal to the latter. The calcium and potassium content of these was as follows:

	Scirrhous carcinoma	Benign papilloma	Ulcerating carcinoma	Mucosa
Calcium (mgm. %)	5.2	4.9	4.4	9.4
Potassium (mgm. %)	282.0	314.0	264.0	144.0

Thus the calcium and potassium content of the benign papilloma was of the same order as that of the 2 separate carcinomas on each side of it, relative to the values observed for the uninvolved mucosa.

TABLE I: ANALYSES OF GASTRIC CARCINOMA AND MUCOSA

	% H_2O *		% Fat, wet tissues		Calcium,* mgm. %		Potassium,* mgm. %		N/Kg, gm.	
Patient	Mucosa	Neoplasm	Mucosa	Neoplasm	Mucosa	Neoplasm	Mucosa	Neoplasm	Mucosa	Neoplasm
1. Web.	85.6	85.6	2.0	0.6	9.9	6.2			20.9	20.3
2. Dav.	85.6	83.4	1.8	1.8	8.2	5.3	187	413	19.3	22.0
3. Pet.	85.6	84.5	0.5	0.6	7.6	6.2		189	19.3	19.4
4. Krum.	82.7	82.9	2.1	3.7	6.7	5.7	234	190	22.0	24.8
5. Dahl.	86.5	85.5	0.14	0.6	7.2	6.2	244	320	20.1	22.3
6. Wo.	86.1	84.2	2.8	0.52	8.6	7.2	214	324	20.9	22.6
7. Ort.	88.5	84.7	0.7	0.01	12.7	12.2	289	322	16.9	24.5
8. Mag.	85.0	83.0	1.7	2.5	8.5	5.5	209	295	21.4	22.9
9. Zil.	86.3	83.6	1.33	1.7			164	184	18.0	21.5
10. Duz.	85.0	83.0	1.8	0.7	7.8	4.9	206	285	20.5	24.7
11. Zig.	87.2	83.2	1.1	2.2	9.7	9.4	170	260	17.2	22.7
12. Mot.	84.0	90.0	5.8	0.9	9.7	8.1	180	325	22.7	22.2
13. Niem.	81.0	83.0	0.5	0.8	11.8	7.7	211	304	20.1	23.1
14. Pear.	80.0	86.0	1.53	0.18	12.4	9.0	205	390	22.2	24.6
Average	84.9	84.5	1.7	1.2	9.3	7.2	209.4	292.4	20.1	22.7

* Calculated on basis of fat-free wet tissue.

TABLE II: ANALYSES OF GASTRIC MUCOSA FROM PATIENTS NOT PRESENTING GASTRIC CARCINOMA

Patient	Diagnosis	% H_2O in fat-free tissue	Calcium,* mgm. %	Potassium,* mgm. %
Doh.	Atroph. gastritis	83.4	8.8	248
Ik.	Atroph. gastritis	81.6	7.4	...
Lalicat.	Hodgkin's disease	85.7	7.2	197
Hostet.	Stoma ulcer	84.8	6.2	187
Moc.	Peptic ulcer	87.8	6.4	...
Damer.	Peptic ulcer	86.5	7.8	...
Eck.	Peptic ulcer	83.7	6.1	238
Average		84.8	7.1	217.5

* Fat-free tissues.

DISCUSSION

In a few instances samples of mucosa adjacent to the neoplasms and at the greatest distance possible from them were analyzed at the beginning of these studies in an endeavor to determine whether or not there was a gradient in the abnormal K/Ca ratio related to the distance from the neoplasm. This was not the case. The abnormal K/Ca ratio observed in the neoplasms is an attribute of them, and there is no evidence to suggest that such a disturbance might obtain in the uninvolved mucosa of cancerous stomachs, a change which, if present, might be interpreted to indicate carcinogenic factors in operation over the entire mucosa.

The results obtained by analysis of the benign papilloma situated between the 2 carcinomas, which showed a K/Ca ratio of the same order as that observed for the carcinomas relative to the uninvolved mucosa, is of interest in view of the general impression that benign papillomas of the stomach are prone to malignant degeneration.

The results observed in 2 instances of lymphosarcoma of the stomach, which parallel those obtained for carcinoma, but in which there was even more pronounced alteration in the K/Ca ratio, indicate that this abnormal ratio is characteristic of neoplasms in general rather than a specific quality of gastric carcinomas, inasmuch as lymphosarcoma is not a neoplastic transformation of tissue peculiar to the stomach.

SUMMARY

Gastric carcinomas were found to contain relatively less calcium and more potassium than the adjacent uninvolved gastric mucosa. In a benign papilloma of the stomach situated between 2 separate carcinomas the K/Ca ratio was of the same order as in the carcinomas, and differed in like manner from the K/Ca of the surrounding uninvolved mucosa.

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Potassium and Calcium Content of Carcinomas and Papillomas of the Colon*

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In the previous communication results were reported to show that the K/Ca ratio in carcinoma of the stomach was increased relative to that in the adjacent mucosa and relative to the mucosa of noncancerous stomachs. This increase was the result of elevated potassium and decreased calcium in the neoplasms.

Similar studies were carried out for carcinomas and papillomas [1] of the colon. The methods of analysis were identical with those employed in the studies on gastric carcinoma and will not be described here.

The results obtained for carcinoma of the colon are summarized in Table I. The average water content of mucosa and carcinoma was approximately the same. In most instances the mucosa contained a greater quantity of ether-extractable fat. The calcium content of the mucosa was greater than that of the carcinoma, malignant neoplasms having a reduced calcium content relative to the normal tissues from which they arise. The increase in potassium content of the carcinoma relative to the mucosa was rather pronounced. The nitrogen content was slightly higher in the neoplasms than in the mucosa.

In 4 instances benign papillomas were present in the resected segments of colon at various distances from the malignant neoplasms. The results of analyses

TABLE I: ANALYSES OF CARCINOMAS OF THE COLON AND ADJACENT UNINVOLVED MUCOSA

Patient	% Water, wet tissue *		% Fat, wet tissue		* Mgm. % calcium		* Mgm. % potassium		Gm. N/Kg. wet tissue	
	Mucosa	Neoplasm	Mucosa	Neoplasm	Mucosa	Neoplasm	Mucosa	Neoplasm	Mucosa	Neoplasm
Crum.	85.1	83.8	0.26	0.02	14.8	10.1	190	362	22.8	23.0
Mast.	87.9	86.8	0.66		14.7	6.3	138	256	18.1	20.6
Sha.	83.1	82.9	0.84	0.31	15.5	7.5	242	379	24.4	23.7
Culle.	85.5	83.0	3.0	1.5	11.1	7.5	200	346	19.6	22.3
Fif.	86.5	85.5	3.6	6.1	8.6	7.8	141	261	19.4	21.9
Gram.	83.2	80.0	9.2	2.5			207	258	20.4	24.9
Fro. (2 cases)	82.7	83.6	2.1	2.9	13.05	10.8	226	345	22.7	23.8
		83.6		1.8		9.6		382		23.0
Stank.	84.2	86.0	10.0	0.6	10.1	8.6	164	219	19.6	23.8
Finitz.	81.0	83.2	8.1	0.93	12.8	2.64	217	348	21.2	21.9
Kin.	85.0	84.1	0.3	2.0	9.5	6.34	173	298	19.6	22.6
Sh.	83.5	82.0	2.5	2.9	10.8	10.0	214	347	23.2	24.3
Average	84.3	83.7	3.69	1.96	12.1	7.9	192	317	21.0	23.0

* Fat-free wet tissues.

TABLE II: ANALYSES OF CARCINOMAS AND PAPILLOMAS OF THE COLON AND OF ADJACENT MUCOSA

Patient	% Water, wet tissue *			% Fat, wet tissue			Calcium,* mgm. %			Potassium,* mgm. %			Gm. N/kgm., wet tissue		
	Muc.	Pap.	Ca.	Muc.	Pap.	Ca.	Muc.	Pap.	Ca.	Muc.	Pap.	Ca.	Muc.	Pap.	Ca.
Schn.	85.4	81.5	83.2	2.00	0.98	1.34	10.9	8.6	6.1	170	351	317	21.0	23.9	22.0
Hanc.	86.5	84.7	85.5	1.40	1.7	1.30	14.0		9.3	174	286	337	18.0		19.8
Gr.	83.2	81.8	83.0	2.70	3.9	2.70	16.7	14.4	7.6	190	295	312	21.3	21.6	22.7
Plu.	84.9	82.5	83.4	1.26		1.37	11.9		7.8	180	308	324			
Average	85.0	82.7	83.8	1.84	2.19	1.68	13.4	11.5	7.7	178	310	323	20.1	22.8	21.5

* Fat-free wet tissues.

* This work was conducted under a grant from the Charles H. and Mary F. S. Worcester Memorial Fund, University of Chicago.

[1] True papillomas, not polyps.

are summarized in Table II. The water and fat content was similar. The calcium content of the mucosa was greater than that of the papillomas and carcinomas; the difference between mucosa and carcinoma was more pronounced than that between mucosa and papilloma. The potassium content of both papillomas and carcinomas was considerably greater than that of the mucosa. The nitrogen content was also slightly higher in the papillomas and carcinomas than in the adjacent mucosa.

DISCUSSION

In comparison with similar analyses carried out on gastric carcinoma and adjacent uninvolved mucosa it is observed that the water content of malignant and nonmalignant colon mucosa resembled that observed in the former. The ether-extractable fat was greater in colon than in gastric carcinoma, and appreciably greater in colon mucosa than in gastric mucosa. The calcium content of the uninvolved colon mucosa was distinctly higher than that of gastric mucosa. This was to be expected in view of the fact that calcium is normally excreted by colon mucosa. The calcium content of colon carcinoma was higher than of gastric carcinoma. The potassium content of uninvolved gastric and colon mucosa was approximately the same, and the degree of increased concentration of potassium in colon carcinoma was approximately the same as the increased concentration in gastric carcinoma. The total nitrogen content per unit weight of gastric and colon carcinoma and mucosa was slightly higher in the neoplasms.

In view of the general impression that papillomas of the colon are precancerous, the fact that their potassium content was of the same order as that of the carcinomas and their calcium content deviated less from the normal prompts the suggestion that actual cancerization is associated with a degree of local reduction in calcium, once the increased local concentration of potassium has been obtained.

SUMMARY

Carcinomas of the colon were found to contain less calcium and more potassium than adjacent normal mucosa. In papillomas of the colon, an increase in potassium of the same order as that found in carcinomas was observed, but the degree of reduction in calcium was not so pronounced as in the latter.

Relationship of the Inherited Susceptibility and the Inherited Hormonal Influence in the Development of Mammary Cancer in Mice*

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It has been demonstrated recently that in addition to determining the inherited susceptibility to mammary cancer in mice, the genes play a role in controlling some phase of either hormonal production and/or metabolism (13, 14, 16, 17). This later effect is discernible primarily in the genesis of mammary cancer in virgin females and is now being called the inherited hormonal influence (13, 24).

This report gives comparative data on the genetic make-up of the inherited susceptibility to mammary cancer and the inherited hormonal influence in different high-cancer strains of inbred mice and their reciprocal hybrids. Although the observations on the inherited hormonal influence are complete for the present study, they should be considered as preliminary data, since further studies will be necessary to determine the genetics and physiology of this influence. Consideration will also be given to a possible difference in the nature of the milk agent as transferred by mice of the two cancerous strains (13).

MATERIALS AND METHODS

All the animals used for this report, with the exception of a small proportion of the F_1 hybrids, were born in Minneapolis. The others were moved from Bar Harbor when they were no older than 3 months of age.

To distinguish easily between mice of the same stock with and without the milk agent, the various lines have been designated as follows:

Aa stock.—Mice of the cancerous A stock that were nursed by mothers with the milk agent. Observations were made on representatives of the 67th to 73rd inbred generations.

Ax stock.—Descended from 1 animal of the A stock that had been nursed by a female of the X or CBA strain (4, 6). The descendants of the 19th to 25th generation since fostering were used to give the present data.

Zz stock.—The cancerous Z or C3H strain, in which the mice possessed the milk agent. Results secured on mice of the 52nd to 57th generations.

Zb stock.—Fostered mice of the Z stock that did not have the milk agent. The mice studied were the descendants of the 9th to 15th generation of 3 fostered females (10).

The origin of the A and C3H or Z stocks has been given by Strong (25).

In describing the mice of the hybrid generations, the maternal strain is always given first: as Aa♀ × Zz♂ + $AaZzF_1$. The mice of the second generation from this cross are called $AaZzF_2$. Thus the capital letters represent the stocks, and the small letter following the maternal strain gives the source of the milk; active milk agent either "a" or "z"; no milk agent would be expected in the mice with either "x" or "b."

Both virgin and breeding females were observed in all the groups. The mice were housed 5 per pen, and received Purina fox chow and tap water. Mice that did not have the milk agent are included only if they survived to 300 days. The incidence of mammary cancer was determined on the number living to the age at which the first tumor was discovered.

EXPERIMENTAL RESULTS

Table I gives the incidence of spontaneous mammary cancer and the average ages for the breeding females of the inbred stocks and their reciprocal hybrids.

Tumors were noticed at an earlier average age in mice of the Zz stock than in the Aa animals. The

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respective incidences were 95.1 per cent and 86.7 per cent.[1]

The incidence of mammary cancer in the reciprocal F_1 and F_2 hybrids with the milk agent that were continued as breeders was, in every group, equal to that observed in the parental stocks. Only 1 hybrid animal survived beyond the average cancer age, and was recorded as noncancerous; this mouse died at 11.5 months. Considering the number of mice that was observed in each group, there was probably no significant variation in the average cancer ages.

No mammary tumors were observed during the course of the study in breeding females of the Ax and Zb stocks and their reciprocal F_1 hybrids. The total number that survived to 300 days of age was 376.

TABLE I: INCIDENCE OF MAMMARY CANCER IN BREEDING MICE OF THE A AND Z STOCKS AND THEIR RECIPROCAL HYBRIDS

Capital letters indicate the stocks, and small letters the source of the nursing influence (active agent represented by either a or z; x or b, no active agent). All the females were used as breeders.

Stock	Generation	No.	% Ca.	Average cancer age	Average non-cancer age
Aa	F67-F73	128	86.7%	10.0 mo.	15.0 mo.
Zz	F52-F57	224	95.1%	9.1	10.4
$AaZzF_1$	Aa♀ × Zz♂ *	51	98.0%	9.7	11.5
$ZzAaF_1$	Zz♀ × Aa♂ *	40	97.6%	10.1	7.5
$AaZzF_2$	F_1♀ × F_1♂ *	46	95.8%	11.2	8.5
$ZzAaF_2$	F_1♀ × F_1♂ *	42	100.0%	10.0	...
Ax	F19-F25 *	139	0.0%	...	14.2
Zb	F9-F15 *	123	0.0%	...	14.0
$AxZbF_1$	Ax♀ × Zb♂ *	51	0.0%	...	18.3
$ZbAxF_1$	Zb♀ × Ax♂ *	63	0.0%	...	18.0

* Generations since fostering

The data for the virgin females of the inbred stocks and the hybrid generations are tabulated in Table II.

As was the case in the breeding females, mammary tumors were not found to occur in any of the virgin females of the inbred stocks and their hybrids that did not have the milk agent.

The incidence of mammary cancer in virgin females of the Aa stock was 3.9 per cent, as contrasted with the incidence of 63 per cent for the virgins of the Zz strain. The F_1 virgins with mothers from the Aa line had a higher incidence (92.5 per cent) than did the hybrids of the first filial generation with maternal parents from the Zz stock, in which 73.2 per cent developed cancer. This difference persisted in mice of the reciprocal F_2 generations, or 70.4 per cent and 38.8 per cent respectively. The average cancer ages were lower in the hybrids descended from females of the Aa stock. Whereas the average cancer age was earlier in the $ZzAaF_2$ hybrids than in the $ZzAaF_1$ mice, this was not the case for the F_1 and F_2 animals of the reciprocal cross. There was no significant variation in the average age at death of the noncancerous mice in the various groups.

TABLE II: INCIDENCE OF MAMMARY CANCER IN VIRGIN FEMALES OF THE A AND Z STOCKS AND THEIR RECIPROCAL HYBRIDS WITH AND WITHOUT THE MILK AGENT

Generations, etc., given in Table I

Stock	No.	% Ca.	Average cancer age	Average non-cancer age
Aa	102	3.9%	15.0 mo.	19.3 mo.
Zz	92	63.0%	13.3	15.9
$AaZzF_1$	93	92.5%	15.2	18.9
$ZzAaF_1$	56	73.2%	19.1	22.5
$AaZzF_2$	98	70.4%	16.2	21.8
$ZzAaF_2$	98	38.8%	17.2	22.0
Ax	44	0.0%	...	18.7
Zb	50	0.0%	...	18.3
$AxZbF_1$	52	0.0%	...	19.1
$ZbAxF_1$	48	0.0%	...	21.6

DISCUSSION

It has been found that virgin females of the cancerous A stock have a low incidence of mammary cancer, whereas the virgin females of the Z or C3H stock have a high incidence. When reciprocal matings are made between mice of these 2 strains, the F_1 hybrid females, when maintained as virgins, have an incidence as high as, or higher than, that observed in the virgin females of the Z stock. In accord with these data it has been postulated (14, 17, 16, 13, 24) that the difference in the incidence for virgin females of these 2 cancerous stocks is due primarily to some gene-controlled hormonal mechanism. This gene-hormone relationship has been termed the "inherited hormonal influence" (13, 24). Although it is of primary importance in the development of mammary cancer in virgin females, it is possible that it may also influence the average age at which breeding females develop cancer (24). Woolley and his group (27-29) described the development of adrenal hyperplasia and the subsequent appearance of mammary cancer in ovariectomized mice of certain strains. Smith (24) has been able to associate these effects with the inherited hormonal influence. The present data are

[1] Female No. 90681 of the Aa strain developed mammary cancer at 403 days of age and was a member of the 46th successive cancerous generation. Ten of her progeny are included in the data, of which only 3 had cancer. Five of these noncancerous mice were sacrificed for use in another experiment at either 533 or 601 days of age; 1 of the cancerous progeny developed cancer at a later age. Nine other descendants, in undepleted litters, were continued, of which only 1 developed cancer; the others have survived for at least 16 months. Further studies are indicated to determine the reason for the change in the incidence in mice of this line; if the mice are not included above, the incidence for the Aa stock was 92 per cent, approaching that observed in the Zz animals.

given to characterize further the genetic aspects of the influence while clarification of the physiological mechanisms involved await future investigation.

Evidence for the segregation of genes determining the inherited hormonal influence was obtained in the A×Z reciprocal crosses by observing the virgin females of the F_2 generations, in which the incidence of tumors dropped sharply from that seen in mice of the F_1 generations. It is to be noted, however, that there is a significant difference in the incidences for the reciprocal groups, those animals of both the F_1 and F_2 generations with A mothers and Z fathers had the higher incidences. According to genetic principles the mice of these reciprocal groups would be expected to have the same genetic constitution. Thus not only would they be expected to inherit the same susceptibility to cancer development, but also the same factors determining the level of hormonal stimulation and the same end-organ sensitivity to the various inciting influences. Hence it seems very probable that a difference in either the concentration and/or activity of the agent in the milk of the A and Z (C3H) mothers was responsible for the variation observed in the incidences for the reciprocal virgin hybrids (13).

Andervont (1) found that the incidence in virgin females of his C3H stock was higher than we observed in our line, and Heston and Andervont (17) noted that when reciprocal matings were made between mice of the A stock and their C3H strain the incidences in the virgin F_1 hybrids were approximately the same, and that reciprocal foster nursing did not significantly alter the incidence of tumors in the various groups. This indicated that there was no appreciable difference in the milk influence in their lines of these stocks. The results published by Warner, Reinhard, and Goltz (26) on the virgin F_1 hybrids produced by crossing mice of the A and M stocks, were, however, more in accord with our observations. The hybrids with A mothers had a higher incidence than did those with M mothers. One of their conclusions was that the milk agent was more concentrated in the A than the M stock.

The observations of Murray and Little (22) and Murray (20, 21) should be cited, although the data were obtained in different laboratories. When the incidence in the virgins of the dilute brown stock was 50 per cent, the hybrids with mothers of this strain and fathers from the C57 black stock showed an incidence of 39.8 per cent (22). When hybrids with C57 black mothers were nursed by females of the dilute brown stock they had an incidence of 61.6 per cent, or higher than was found in the other group. However, the virgin females of the dilute brown stock, in the meantime, had an increased incidence, or 61.5 per cent. While these observations may not be comparable, the increased incidence in the cancerous strain might have resulted from a change in the nature of the milk agent, but other explanations also are possible.

In previous reports (11-13) data obtained on breeding females resulting from crosses of the A and B (C57 black) stocks were interpreted according to the theory that the genetic susceptibility for mammary cancer, as transmitted by mice of the cancerous A stock, is probably determined by multiple factors, one of which is linked with the gene for brown coat color. In the present cross the incidence in the virgin F_1 and F_2 hybrids with mothers from the cancerous A stock was approximately the same as in the breeding females of similar generations of the A×B cross.

While the data are not adequate to indicate the number of genes needed to produce the inherited hormonal influence, it appears probable that multiple factors are involved. Genetic analysis will be difficult, however, since the incidence in mice of the hybrid generations may be influenced by possible differences in the characteristics of the milk agent as transferred by mice of the 2 cancerous stocks. Reciprocal foster nursing experiments are being conducted in order to obtain information concerning these points.

The reciprocal F_1 and F_2 hybrids between the cancerous A and Z stocks, when they were maintained as breeders, showed the same incidence that was found in breeding females of the 2 parental stocks. These results were the same as when either mice of the 2 lines (cancerous x fostered) of the A or the 2 lines of the Z stocks were tested; that is, mice with the same inherited susceptibility for mammary cancer (8-10). Thus it would seem probable that the mice of the A and Z strains may have the same susceptibility to mammary cancer. If different genes were transmitted, segregation of these might not be evident in mice of the F_2 generations, as any combination might produce the susceptibility; but it should be noted in mice of the succeeding generations. None, however, has been found in animals of the F_3 to F_6 generations.

If the genes responsible for the inherited susceptibility to mammary cancer are identical in animals of the A and Z stocks, it would follow that either a separate gene or different genes are transmitted to produce the inherited hormonal influence, since only mice of the Z stock transferred it. However, since multiple factors are probably involved in each of these effects, it is possible that one or more genes may take part in the determination of the susceptibility and the hormonal influence. The relationship between the genetic make-up of the 2 effects must wait until other studies have been completed.

Any variation in the characteristics of the milk agent of the 2 parental stocks was not apparent in the

reciprocal hybrids when they were maintained as breeding females. This would suggest that following the increased hormonal stimulation, either the tissues were more sensitive or the amount of the agent had increased. If, however, there may be a considerable difference in either the incidence and/or the average cancer age in breeding females of 2 cancerous stocks, the maternal influence may be noted in the reciprocal F_1 hybrids even when they are used as breeders (3).

Virgin and breeding females of the fostered A and Z stocks and their reciprocal F_1 hybrids remained free from mammary cancer. These mice had the inherited susceptibility for the development of spontaneous mammary cancer and, in addition, the animals of the Zb stock and the reciprocal F_1 hybrids would be expected to have the inherited hormonal influence. An increased hormonal stimulation would occur in the females that were bred. As mammary tumors were not observed in any mouse of these various groups the lack of the milk agent, even in susceptible animals with an adequate hormonal level, was completely determining in its effects.

Recently Warner, Reinhard, and Goltz (26) have estimated the relative importance of the milk agent as compared with the inherited susceptibility in the genesis of mammary cancer in mice as follows: "The inherited susceptibility of the physiological system is of greater importance than the milk agent in the development of mammary cancer in these 2 strains of mice." The authors stated, however, that their conclusions would apply only to their data. In considering an interpretation of their results, the possible role of the hormones in the etiology of mammary cancer was not cited. Their observations, obtained by crossing mice of the cancerous A and M stocks, were, with the exception of the incidence in the virgin females of the A stock, approximately the same as ours for the cross between the A and Z stocks. While they found an incidence of 29 per cent in virgin females of the A stock, we observed only 3.9 per cent, and in no other study has it exceeded 5 per cent (Loeb and Kirtz, 19; Bittner, 7; and Heston and Andervont, 17). No possible explanation for this incidence was suggested by Warner and his associates. The main difference in the interpretation of the 2 sets of data was that we have recognized, in accordance with conclusions published previously (14, 17, 16, 13, 23), the role of the genes in determining the inherited susceptibility to mammary cancer as a different physiological effect from what we have called the inherited hormonal influence. The transmission of the inherited hormonal influence by animals of the M stock would account for the incidence of 98 per cent that was observed by Warner and his group in the virgin hybrids with mothers from the A stock. It is possible, because of the incidence found in virgins of the A stock, that some animals of their line also may have had the inherited hormonal influence, since it has been separated from our strain for several years. Thus our interpretation need not apply only to the present data but to other studies as well.

No critical data have yet been published that would suggest any difference in the relative importance of the role of the milk agent, inherited susceptibility, and hormonal stimulation in the genesis of mammary cancer in mice. Interpretations intimating such a difference have either been modified after experiments on reciprocal foster nursing were completed (20-22), or else all the primary inciting influences have not been taken into consideration (26). Our conclusions (5, 9) are similar to those of Heston (16), who stated recently: "Mammary tumors do not result from any of these factors or sets of factors, but from the action and interaction of all three, and it would be folly to attempt to say which is the most important." Also, as far as can be determined at the present time, the genesis of mammary cancer is probably the same in all strains of mice and may be explained on the same interpretation of the data.

In addition to the inherited susceptibility for mammary cancer and the inherited hormonal influence, other inherited physiological effects have been described, which may or may not have some role in the development of mammary cancer. Shimkin and Andervont (23) injected estrogens into ovariectomized mice and found that the degree of response of the genital organs was determined by the genetic constitution of the animals. These findings have been confirmed, and it has been noted that the sensitivity of the genital organs may be transmitted by a genetic make-up that may be different from either the inherited hormonal influence or the inherited susceptibility for mammary cancer (Samuels and Bittner, unpublished data). Heston, Deringer, and Andervont (18, 15, 2) have suggested also that still another set of genes may perhaps function in the propagation and transmission of the milk agent.

Thus, as our information becomes more extensive, the "physiological system" must be separated into individual effects, each probably controlled by the action of a relatively small number of primary genes. Whether or not some of these effects may play a role in the genesis of mammary cancer must be determined by further studies.

CONCLUSIONS

During the course of this study mammary tumors were not found in any of the descendants of the *fostered* females of the cancerous A and Z (C3H) stocks and their reciprocal hybrids, mice that were suscep-

tible to mammary cancer but did not have the milk agent.

Observations obtained on breeding females of the cancerous A and Z stocks and their reciprocal F_1 and F_2 hybrids were in accord with the theory that the mice of these 2 strains may have the same inherited susceptibility to mammary cancer.

The difference in the incidence of mammary cancer in the virgin females of the A and Z strains was due to gene action in controlling some hormonal mechanism. This effect has been called the inherited hormonal influence, and is probably the result of the action of multiple genes.

The same genes probably do not produce the inherited susceptibility and the inherited hormonal influence.

The characteristics (concentration and/or activity) of the milk agent may differ in mice of the A and Z stocks and influence the incidence of mammary cancer that was observed in the hybrids when they were maintained as virgins. These differences were not apparent in the breeding hybrids.

Each inciting influence in the genesis of mammary cancer in mice may be nearly completely determining in its effects, and thus equally important.

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A Comparative Morphological Study of the Mammary Glands with Reference to the Known Factors Influencing the Development of Mammary Carcinoma in Mice*

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INTRODUCTION

Early in the study of mammary carcinoma in mice attention was given to the correlation of mammary gland structure and the occurrence of malignant growth. Haaland (31), in a rather extensive study, emphasized the frequency with which small adenomatous areas, previously described by Apolant (6), were encountered in the glands of mice with mammary carcinoma as compared with those of mice dying without cancer. That these localized areas of alveolar hyperplasia, occurring in otherwise atrophic glands, did not represent developmental malformations was indicated by their absence in mammae of young animals, and their precancerous nature was established by demonstrating transitional stages between them and frank carcinoma. It was pointed out further that the hyperplasia was not dependent upon pregnancy, as it occurred in virgin mice; but it was felt that the lesions were related to inflammatory changes caused, perhaps, by the presence of nematodes within the glands.

The development of highly inbred strains of mice represented a definite refinement in the study of mouse mammary carcinoma and made possible the study of glandular alterations preceding cancer formation, for now it could be predicted with relative certainty that a given mouse would or would not develop mammary cancer later in life. Gibson (28) compared the life history of the mammae of two strains of albino mice, one a low tumor the other a high tumor strain. She observed a tendency toward anomalous nipple formation and a generally slower glandular development in the mice of the high-tumor strain. Also, carcinomas were seen to form "in zones of chronic cystic mastitis," but these areas apparently differed from the small adenomas discussed by Haaland and Apolant, for they were described as ducts filled with inspissated secretion and surrounded by a lymphocytic infiltrate. Gardner and Strong (26) extended the investigation of developmental differences by studying the glands from virgin mice of 10 inbred strains, but could demonstrate no correlation between the occurrence of developmental abnormalities or the rate of glandular development and the incidence of mammary carcinoma. As the oldest mice included in their investigation were only 100 days of age, neither adenomatous nor chronic mastitic changes were encountered.

Fekete (21) compared the mammary histology of the high cancer dba and the low cancer C57 black strains, following the glandular development and regression through successive pregnancies. She noted "abnormal areas" in the glands of the dba mice from the time of the second pregnancy until the development of mammary carcinoma. Histologically these "abnormal areas" corresponded closely to the small adenomas described by both Haaland and Apolant, consisting of a compact group of alveoli, often containing numerous mitotic figures, and surrounded by an increased amount of connective tissue. It was further noted that during the latter part of pregnancy and throughout the period of lactation these alveoli did not respond as did the normal tissue, but remained in a proliferative phase without progressing to milk production. Following the cessation of lactation, areas of subinvolution were also noted in the glands of the dba mice.

Gardner, Strong, and Smith (27) gave particular attention to the occurrence of these localized areas of alveolar development in glands of multiparous mice of 5 strains with varying incidence of mammary carcinoma. They were found to be most frequent in the glands of mice from the 3 strains exhibiting a high incidence of spontaneous tumors, whereas they were

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rarely encountered in the very low tumor strain. Furthermore, they were found to increase in number as the animals increased in age, but were independent of the stage of the estrous cycle or the period of time elapsing after the cessation of lactation. The precancerous nature of the lesions was stressed, but in spite of their having been produced experimentally by the administration of estrogenic substances (25, 29, 44) it was stated that factors other than hormonal were also of importance in their development. Further evidence of the precancerous nature of these nodular hyperplasias was given by Gardner (24), who demonstrated that a certain percentage of such lesions, when transplanted into hypophysectomized mice of the same strain, grew progressively and thus seemed to be autonomous under such conditions. The relationship of these nodules to mammary carcinogenesis has also been emphasized by Taylor and Waltman and by van Gulik and Korteweg, so that their precancerous nature has been firmly established and very generally recognized.

The important role played by extrachromosomal influences in the development of mammary cancer was reported independently in this country in 1933 (38) and in the Netherlands in 1934 (36), and followed shortly by the demonstration of the "milk influence" (9). The relationship of such factors to mammary architecture was investigated by van Gulik and Korteweg (42). Employing principally virgin mice of the high-tumor dba and the low-tumor C57 black strains and their reciprocal crosses, and foster nursing some of the offspring of each strain, they felt that 3 factors influencing the definitive structure of the mammae could be distinguished. The configuration of the primary duct was found to be that of the maternal parent regardless of other factors and was said, therefore, to be dependent upon an "influence of the plasma and/or uterus"; the pattern formed by the main ducts of the gland varied according to the chromosomal constitution of the animal, being influenced almost equally by the male and the female parent; the degree to which small lateral buds formed along the ducts of the glands was dependent upon the type of milk the animal obtained during nursing, a greater amount of budding occurring in animals that obtained the milk influence. Further, through comparison of the incidence of mammary tumors with the several variations in mammary structure occurring in the different groups of mice, they concluded that "The degree of the disposition for cancer of the mammary gland is closely connected with the anatomical architecture of this organ." In the following year Shimkin, Grady, and Andervont (37), nursing low tumor C mice on high-tumor C3H foster mothers, confirmed van Gulik and Korteweg's observation that the presence of the milk influence increased the degree to which lateral buds formed along the mammary ducts.

Following this lead an experiment was set up in this laboratory (34) to determine how early the increased lateral budding would appear in the glands of suitable test animals after injection of the milk influence, in an attempt to develop a more rapid test for the presence of active agent in treated tissue extracts. Four week old, low tumor, ABC virgin mice were injected with an extract of mammary gland containing the milk influence, and for the next nine months their mammae were compared at intervals with those of litter mate controls that had not received such an injection. Although some variation in the amount of lateral budding was noted, no consistent difference could be demonstrated between the glands of the mice that had received the milk influence and the glands of those that lacked it. This negative finding, coupled with the finding of a small number of precancerous nodules in virgin C3H mice that had been maintained in anestrus by dietary restriction, and in which no tumors had developed (35), led us to make the present more extensive investigation.

According to our present concept (11, 14) the development of mammary cancer in mice is dependent upon 3 main factors: the genetic constitution of the animal, the hormonal stimulation to which the mammae are subjected, and the presence of a virus-like agent transferred to the offspring through the milk of the nursing female.[1] It seemed advisable, therefore, to reinvestigate the problem of mammary structure and carcinogenesis, attempting to ascertain the effect of each of these 3 established factors upon the definitive mammary gland architecture. In view of the findings of previous investigators outlined above, it would appear that particular attention should be given to (a) the conditions under which the precancerous areas of alveolar hyperplasia occur, in order to determine the etiologic agents responsible for their spontaneous development; and (b) the factors that influence the degree to which lateral buds develop along the ducts of the mammary glands.

MATERIAL AND METHODS

Groups of animals with varying combinations of the 3 factors were selected for study. Mice of the C3H or Z strain and their F_1 hybrids with the A strain possess all 3 when maintained as virgins, as well as when allowed to breed (16, 18, 32). Those of the A strain, although genetically susceptible and possessing the

[1] A possible "uterine factor" (22, 16) has not been considered in these studies, and it is felt by the workers in this laboratory that the mechanism by which dietary restriction reduces the incidence of spontaneous mammary cancer is primarily hormonal in nature (35).

milk influence, have not the correct hormonal constitution to develop a high incidence of mammary cancer when kept as virgins, although 87 per cent of breeding females are so afflicted. Low cancer Z and A stocks of mice have been developed by fostering one generation of young to females of low cancer strains (13, 15). The Zb strain was originally nursed by C57 black foster mothers and subsequently has been bred brother-to-sister for 15 generations, while the Ax strain was foster nursed by a CBA female and continued by brother-to-sister breeding for 25 generations. In neither of these strains, nor in hybrids between them, has spontaneous mammary cancer been observed in the last 3 years, during which time the material for this study was collected. For animals genetically resistant to mammary carcinoma the C57 black strain was employed. In order that the milk influence should be present they were foster nursed by A strain females, and to insure a sufficient hormonal stimulation they were forced bred (7, 23). In spite of these 2 added factors, the milk influence and an excessive hormonal stimulation, only 10.3 per cent of 58 mice developed mammary cancer.

The groups of mice used in this study may be summarized as follows:

1. Possessing all 3 factors and giving a high incidence of mammary cancer: virgin females of the Z strain and F_1 hybrids between the Z and A strains; breeding females of the A and Z strains, and F_1 hybrids between them.

2. Lacking only the milk influence and giving a very low incidence of mammary cancer: mice indicated in Group 1 except lacking the milk agent, these low tumor strains being designated as Zb and Ax.

3. Lacking only the proper hormonal stimulation and giving a low incidence of mammary cancer: virgin females of the A strain.

4. Possessing the milk influence and an excessive hormonal stimulation, but genetically resistant to the development of mammary cancer and giving a rather low tumor incidence: foster nursed, forced bred C57 black females.

5. Lacking both the correct hormonal stimulation and the milk influence, and giving a very low incidence of mammary cancer: virgin females of the Ax strain.

All mice used were maintained in this laboratory, and were born within a 3 year period. The animals were housed in wooden boxes, fed Purina fox chow checkers *ad libitum,* and had tap water continuously available. They were killed at various ages from 130 to 700 days and their mammae studied histologically by means of both the whole mount and section technics.

The incidence of carcinoma and the average tumor age in the various groups during this period are given in Table I.

TABLE I

		Breeding				Virgin			
Stock	Generation	% Cancer	% Ca.	Average cancer age	Average non-cancer age	No.	% Cancer	Average cancer age	Average non-cancer age
A	67-73	128	86.7	10.0 mo.	15.0 mo.	102	3.9	15.0 mo.	19.3 mo.
Ax	19-25	139	0.0	...	14.2	44	0.0	...	18.7
Z	52-57	224	95.1	9.1	10.4	92	63.0	13.3	15.9
Zb	9-15	123	0.0	...	14.0	50	0.0	...	18.3
AZF_1		51	98.0	9.7	11.5	93	92.5	15.2	18.9
ZAF_1		40	97.6	10.1	7.5	56	73.2	19.1	22.5
$AxZbF_1$		51	0.0	...	18.3	52	0.0	...	19.1
$ZbAxF_1$		63	0.0	...	18.0	48	0.0	...	21.6

These data include all mice of high cancer stocks that reached age at which first tumor was noted in each group, and all mice of low tumor lines that lived to be 300 days of age or older. For Ax and Zb lines figures in "generation" column designate number of generations that these lines have been carried since they were foster nursed, the generations of inbreeding from the genetic standpoint being approximately the same as for corresponding high tumor lines.

To prepare whole mounts of the mammary glands, the skin was slit up the dorsum and carefully removed so that the mammae were left intact and adherent to the removed skin. This was then spread and pinned, under moderate tension, in paraffin pans and covered with Bouin's solution. After 8 to 24 hours of fixation the specimens were washed in running tap water for an equal period and the mammae then removed from the skin under a dissecting microscope. The glands were dehydrated through alcohols and the fat removed in xylene. After rehydration in alcohols and water, they were stained with Mayer's hemalum and again placed in 80 per cent alcohol. In this medium the thoracic glands, numbers 2 and 3 bilaterally, were separated from the panniculus carnosus, and the excess connective tissue was dissected away under a binocular microscope. Differentiation was then carried out in acid alcohol and the blue coloring again brought out by placing the glands in a 0.5 per cent solution of ammonium hydroxide in 70 per cent alcohol. They were then dehydrated in alcohols, cleared in methyl

salicylate, and mounted under glass coverslips in Clarite.

After staining, the posterior glands were inspected under the binocular microscope. Interesting areas were removed, imbedded in paraffin, sectioned at 5 micra, and stained with Delafield's hematoxylin and eosin. In this way the gross structure of the glands could be studied *in toto,* and the more exact histology of interesting structures ascertained by the more conventional histological technic.

EXPERIMENTAL RESULTS

The incidence of tumors in the various groups of animals used for this study is given in Table I. As stated in the preceding paper (17), the development of mammary cancer in virgin mice of strains that possess the milk influence and a genetic susceptibility to the development of this type of neoplasm is dependent upon a genetically determined hormonal constitution. This factor, called the "inherited hormonal influence," appears to be transmitted as a dominant characteristic, for the incidence of tumors in virgin F_1 hybrid mice between the A and Z stocks is equal to, or greater than, that observed in virgin mice of the high tumor parental Z strain. Of extreme interest, moreover, is the difference in both the final incidence of tumors and the average tumor age in virgin AZF_1 and ZAF_1 hybrid mice. These 2 groups of animals, which inherit the same chromosomal constitution, develop significantly different incidences of mammary cancer, the AZF_1 hybrids developing a higher incidence at a younger average age. Yet in spite of this difference in the rate and time of tumor development in the ZAF_1 and AZF_1 hybrids, no significant histological differences could be found in the mammae of the two groups, so that throughout the rest of this paper these reciprocal hybrids will be considered together.

Duct Patterns and Intraductular Concretions

Although the pattern formed by the larger ducts of the mammary gland has no apparent bearing upon the development of cancer, it is of some interest from a genetic standpoint. The architecture in the two principal strains used in this study need not be described in detail; suffice it to say that they are very noticeably different, as can be seen by comparing Figs. 6 and 7. These patterns, however, are remarkably constant from animal to animal within the strains, and are not altered by breeding or by the presence of the milk influence. The glands of the F_1 hybrids between these strains develop a duct pattern intermediate between those of the parental strains, and no difference can be detected whether the female came from one or the other strain. These findings agree completely with those of van Gulik and Korteweg (42), and emphasize the fact that the pattern formed by the larger ducts is determined by genetic factors. No study regarding the factors determining the configuration of the primary duct could be carried out on this material, however, as there was little, if any, difference in the structure of this duct in mice of the A and Z strains.

Concretions of eosin-staining material, probably inspissated secretion, have been noted in the mammary ducts of mice (23, 40), and it was sought here to determine if the occurrence of this abnormality was correlated with the development of mammary cancer. A check of our material revealed, however, that such intraductular concretions were present only in the mice of the Z strain; *i.e.,* the high tumor C3H line. They were about equally common in both virgin and breeding females of this line, but were completely absent from the glands of the low tumor Zb mice as well as from both the high and low tumor lines of strain A and hybrid mice. Thus the occurrence of this alteration could be only partially correlated with the development of tumors, or with any of the 3 factors important in mammary carcinogenesis, and no light could be shed upon etiologic factors responsible for the development of these concretions.

Lateral Buds

From the walls of the mammary ducts of virgin mice protrude rounded evaginations from which clusters of alveoli develop during pregnancy or pseudopregnancy. Because of their position along the sides of the ducts they are referred to as lateral buds, but according to more classical histological nomenclature probably correspond to intralobular ducts (41). They are of particular interest in such a study as this because the degree to which they develop has been correlated with the presence of the milk influence, and thus with the incidence of mammary carcinoma (37, 42). From studies of the literature and the present material, however, it becomes evident that 2 other factors, the age of the animal and the stage of the estrous cycle, had to be considered before observations with regard to the effect of the milk influence could be made.

In both rats and mice variations in the lateral buds, as well as in the end buds, have been correlated with the stage of the estrous cycle by several investigators (19, 20, 26, 39, 41, 45). Turner and Gomez (41) state, however, that although these changes are very noticeable during the first few estrous cycles, they are only slight after that time. Gardner and Strong (26) observed such cyclic changes only during growth of the gland; *i.e.,* until the animals were 60 to 70 days of age; alterations after this time consisted merely of slight ductular dilatation with estrus. However, to be certain that this factor was not important in the consideration

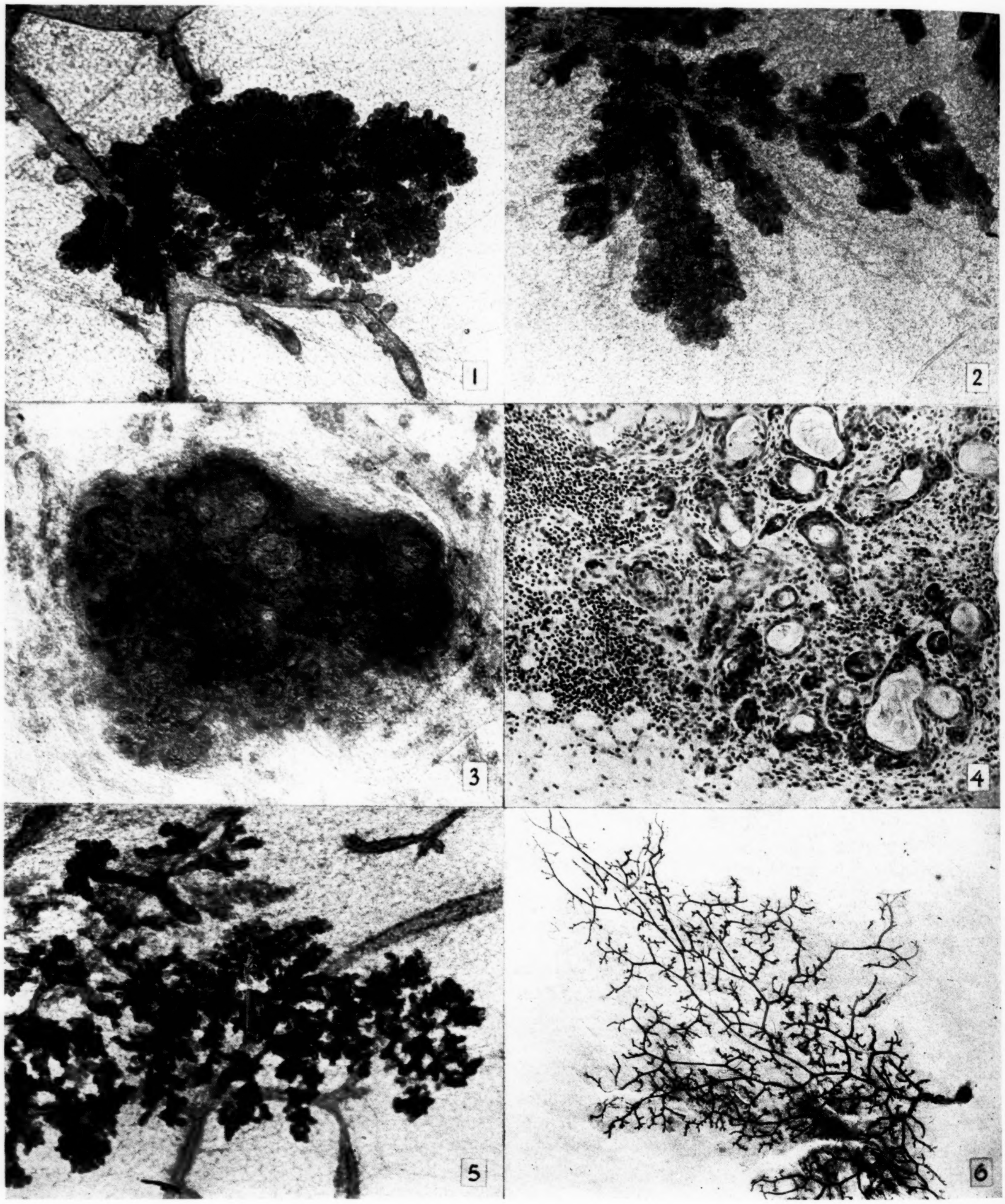

FIG. 1.—Compact alveolar hyperplasia in whole mount preparation. A cluster of abnormal alveoli that have arisen from small area of single moderate-sized duct. Mag. $\times$ 35.5.

FIG. 2.—Area of subdivision in gland of breeding Z mouse. Alveoli not distended with fluid, nor do they appear as distinct from surrounding tissue as do abnormal ones shown in Fig. 1. Mag. $\times$ 35.5.

FIG. 3.—Inflammatory nodule in whole mount preparation. Multicrescentic patterns formed by areas of squamous metaplasia; generally hazy outline of nodule due to extensive inflammatory reaction about it. Mag. $\times$ 35.5.

FIG. 4.—Inflammatory nodule similar to one in Fig. 3, but prepared from section. Note inflammatory reaction throughout area, and prevalence of squamous metaplasia of glandular epithelium. Mag. $\times$ 126.

FIG. 5.—Area of loose, alveolar hyperplasia in whole mount preparation. Here abnormal elements arise from at least 2 different ducts of moderately large caliber. Mag. $\times$ 35.5.

FIG. 6.—Anterior thoracic gland of virgin strain A mouse 248 days of age. Note lack of alveolar nodules and general paucity of lateral buds throughout gland. Mag. $\times$ 4.

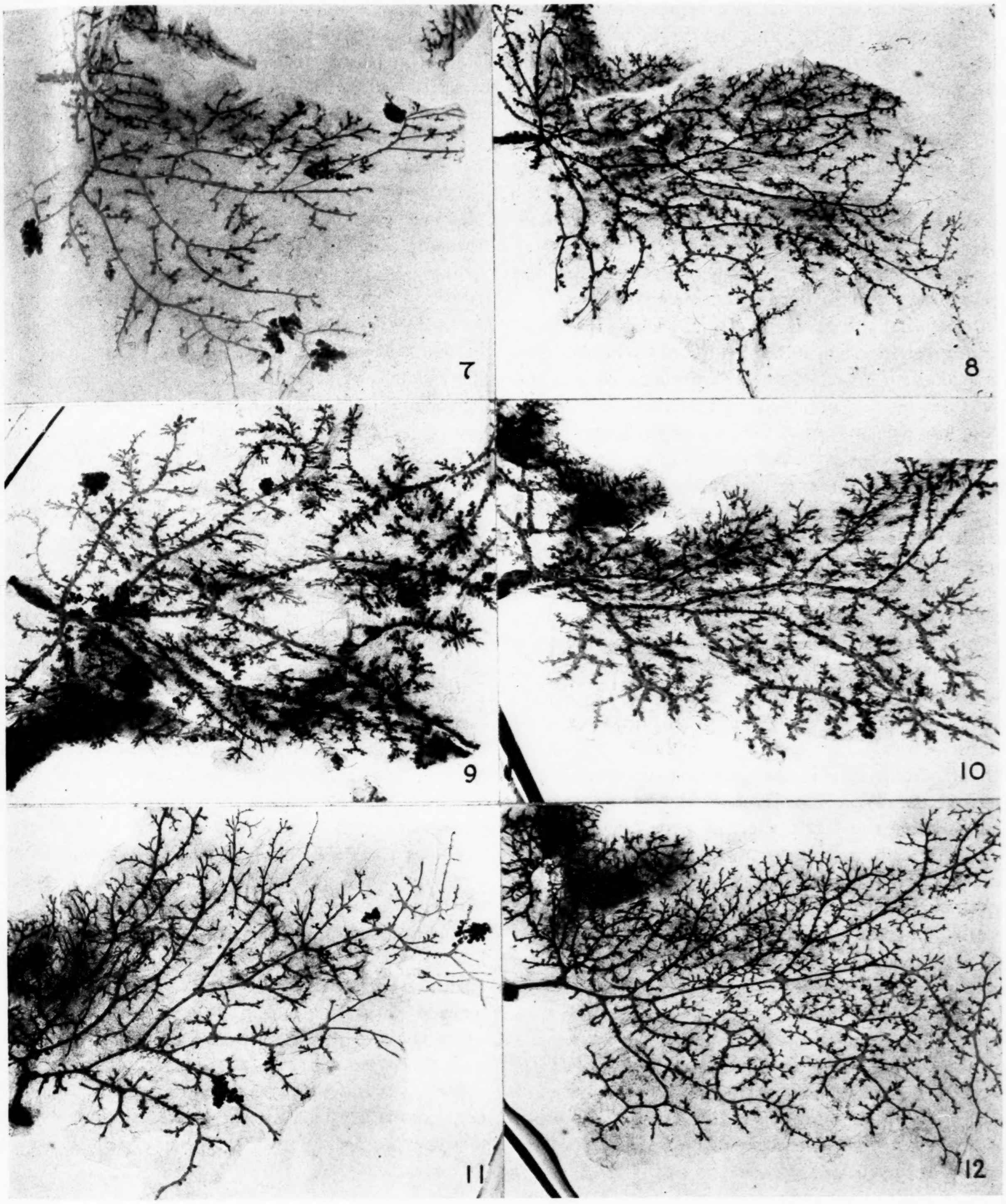

Fig. 7.—Whole mount preparation of anterior thoracic gland of virgin Z female 300 days of age. Note precancerous areas throughout gland. Mag. × 4.

Fig. 8.—Whole mount preparation of anterior thoracic gland of low-tumor virgin Zb female 326 days of age. No areas of alveolar hyperplasia, but intralobular ducts at least as prevalent as in gland of virgin Z mouse in Fig. 7. Mag. × 4.

Fig. 9.—Anterior thoracic gland of breeding Z female 430 days of age. Precancerous areas of alveolar hyperplasia numerous. Mag. × 4.

Fig. 10.—Anterior thoracic gland of breeding Zb mouse 448 days of age. Although there are some areas of moderate subinvolution, there are no precancerous alveolar nodules. Mag. × 4.

Fig. 11.—Whole mount preparation of anterior thoracic gland of virgin ZAF_1 hybrid female 485 days of age. Mag. × 4.

Fig. 12.—Anterior thoracic gland of virgin $ZbAxF_1$ female 451 days of age. Note complete lack of hyperplastic nodules, and yet relative frequency of lateral buds. Mag. × 4.

of the present material, vaginal smears were taken in small groups of Z, Zb, and hybrid mice just prior to the time of sacrifice. The ages of the mice within each group were approximately the same, and the average ages of the groups ranged from 265 to 425 days. When the degree of lateral budding was compared within such groups, the age and strain being constant, no variation according to the stage of the estrous cycle could be noted. Hence it was concluded that because of the age of the mice used in the present study the stage of the estrous cycle at the time of autopsy need not be considered further.

In observing the glands of the virgin Z mice, however, a great variation in the degree of lateral budding was evident. This could not be correlated particularly with the presence or absence of a mammary tumor at the time of autopsy. On the other hand, when the animals were distributed according to age, and the degree of lateral budding arbitrarily graded, 0 designating few buds and 4 profuse budding, a more orderly pattern was seen (Table II). From this somewhat meager series of animals it would appear that the larger ducts of the younger animals are well supplied with intralobular ducts, which then tend to disappear, for during the age period from 200 to 300 days the larger ducts are almost devoid of such elements. After this, however, the number of lateral buds again increases until they become very numerous in the glands of most of the older mice. This tendency to increase with advancing age is even more noticeable in the glands of the oldest Zb mice studied, and suggests the possibility that the development of a mammary tumor, with its effect upon the general health, and more particularly upon the hormonal status (1) of the animal, may influence the development or maintenance of these lateral buds.

A consideration of the relationship of age to the extent of lateral budding in the hybrid mice used in this study did not yield similar results. In this group of mice there is relatively less variation from animal to animal, there being no period when the ducts are almost naked nor when there is the abundance of budding seen in young or older Z mice. In other words, the degree of lateral budding is fairly constant throughout the entire age period from 157 to 717 days, with but a slight tendency to increase with advancing age. In general the same is true of the A mice, although in this group intralobular ducts are much less frequent at all times than in either the Z or the hybrid mice.

With the results of these preliminary inquiries in mind, the question of a relationship of the milk influence to the degree of lateral budding in virgin glands may be considered. When the glands of the C3H mice with (Z) and without (Zb) this agent are compared, no significant difference so far as the number of intralobular ducts present can be seen (Table II). If anything, instead of a decrease, as previously described (37, 42), the glands of those animals lacking the influence seem to be somewhat better supplied with lateral buds than do those of the mice possessing this agent. However, as mentioned before, the presence of a tumor may have some effect in decreasing these elements. In the virgin hybrid mice studied, where the variation from animal to animal within a given group is much less and the two groups overlap with respect to age to a greater extent, absolutely no difference in the degree of lateral budding could be found between the mice having and those lacking this factor. The same is true of the A and Ax groups, although here there is a point of difference, for both have a low-tumor incidence.

To carry the inquiry further the glands of the breeding mice were compared from this standpoint. Here, however, the situation is somewhat complicated by pregnancy and subsequent involution. The glands of the breeding mice were obtained when the animals were not pregnant, and at least one month after the cessation of lactation. However, the females had not been separated in every instance from males during this period, so that the occurrence of a pseudopregnancy cannot be ruled out. Lateral projections from the larger ducts in multiparous females are much more numerous than in virgins, and may consist of both involuted intralobular ducts and subinvoluted alveoli. Contrary to the situation in virgin mice, these lateral projections tend generally to decrease with advancing age; *i.e.,* after about 250 days, in all groups of mice studied. When this is kept in mind, and the gland of the high-tumor lines, that is, those possessing the milk influence, are compared with those of the low-tumor line of the same genetic constitution but lacking this agent, no difference in the number of these lateral projections could be detected. In this material, therefore, when the age and the genetic constitution of the animals compared were the same, the presence of the milk influence was found to have no influence upon the degree to which lateral buds developed along the ducts of the mammary glands in either virgin or breeding animals.

Hyperplastic Lesions

In studying the present material, 3 types of lesions involving the glandular epithelium were encountered: areas composed primarily of hyperplastic alveoli in otherwise resting glands, areas of extensive proliferation of fine ducts, and areas comprised of a few hyperplastic alveoli surrounded by a considerable inflammatory reaction.

The first of these lesions is the typical precancerous

TABLE II

	Age, days	No. of glands	Alveolar nodules	Alv. nod. per gland	Duct nodules	Inflam-matory nodules	Lateral budding
			Z FEMALES (HIGH TUMOR)				
Virgin [18]	130	4	0	0	0	0	2
	130	4	2	½	0	0	1-2
	131	4	0	0	0	0	2
	197 T	4	10	2½	0	0	1
	250 T	4	2	½	0	0	0
	259	3	1	⅓	0	0	0
	259	4	2	½	0	0	0
	287 T	4	13	3¼	0	0	0
	299 T	4	17	4¼	0	0	2
	300 T *	4	26	6½	0	0	1
	301 T	4	66	16½	0	0	1
	308 T	4	17	4¼	0	0	2-3
	337	4	20	5	0	0	1
	343 T	4	26	6½	0	1	0-1
	343	4	39	9¾	0	0	2
	396	4	28	7	0	0	1-2
	398 T	4	51	12¾	0	0	2
	419 T	4	51	12¾	0	0	1
Breeding [11]	135	4	8	2	0	2	
	135	4	22	5½	0	0	
	211 T	4	10	2½	0	1	
	257 T	4	82	20½	1	0	
	261 T	4	102	25½	1	0	
	262 T	4	28	7	0	0	
	312 T	4	34	8½	1	4	
	344 T	4	95	23¾	0	6	
	351 T	4	30	7½	0	5	
	363 T	4	48	12	0	4	
	430 T *	4	66 (2 CA)	16½	0	1	
			ZB FEMALES (LOW TUMOR)				
Virgin [6]	299	4	0	0	0	0	1-2
	304	4	0	0	0	0	1-2
	326 *	4	0	0	0	0	2
	330	4	0	0	0	0	2
	418	4	0	0	0	0	2-3
	434	4	0	0	0	1	4
Breeding [12]	211	4	0	0	0	3	
	235	4	0	0	0	0	
	308	4	0	0	0	2	
	366	2	0	0	0	0	
	371	4	(1)	¼	0	0	
	379	4	1	¼	0	4	
	385	4	0	0	0	2	
	385	4	0	0	0	2	
	448	4	(1)	¼	0	0	
	448 *	4	0	0	0	3	
	448	4	(1)	¼	0	3	
	537	4	(1)	¼	0	2	

Data concerning Z and Zb animals included in this study. "T" after age indicates animal with mammary carcinoma at autopsy; asterisk in "Age" column signifies that one of the glands of the animal is pictured in a photomicrograph. "CA" in parentheses in "Alveolar Nodule" column indicates microscopic carcinoma in one of the glands; figures within parentheses in this column denote presence of increased number of macrophages in otherwise typical alveolar nodule.

alveolar nodule described previously by several authors (6, 27, 21, 31, 40), so that only a few additional words are necessary here. The primary alteration in such a lesion is alveolar hyperplasia, with but slight to moderate increase of the supporting connective tissue and no appreciable evidence of inflammatory reaction. The alveoli are often somewhat distended with inspissated secretion, their epithelium frequently exhibits appreciable mitotic activity, and the surrounding layer of collagenous connective tissue is somewhat thicker than is normally seen. In some of these areas, particularly in the glands of multiparous mice, macrophages filled with a yellowish pigment are rather numerous. However, as these are common in normal areas of the glands of such mice they cannot be considered as an inflammatory reaction to the alveolar hyperplasia. Although these lesions are usually composed of closely packed alveoli (Fig. 1), areas are seen occasionally in which there is considerable space between the abnormal elements, giving rise to a very loose appearing nodule (Fig. 5). Frequently such a lesion contains abnormal lobules that arise from several adjacent ducts, prompting speculation on the mechanism by which alteration of an entire area, as opposed to the focal compact nodules, was brought about. However this occurred, there can be little question that frank mammary carcinomas develop in areas of both compact and loose alveolar hyperplasia, for all gradations between these hyperplasias and carcinoma can be found.

In virgin mice these lesions are very easy to identify, even when they are composed of only a few abnormal alveoli, because normally alveolar elements are not encountered in the virgin gland (26, 41). In the glands of breeding animals, particularly before the involution of advancing age has become pronounced, lobules of subinvoluted alveoli are rather frequently encountered. These differ from the precancerous hyperplasias in that their alveoli are generally smaller, containing little secretion if any, and the mitotic activity often seen in areas of hyperplasia is absent. Furthermore, there is not the increase of supporting connective tissue seen in the precancerous lesions, so that the areas of subinvolution tend to be less distinctly marked off from the surrounding glandular tissue than is the case in the other condition. A comparison of Figs. 1 and 2 will bring out these differences more clearly.

The second type of glandular abnormality encountered is one of ductular proliferation, described previously by Gardner (24). These lesions occur but infrequently, for only 13 were found in the present material. They consist of an area in which there has been an extensive proliferation of very fine ducts. These radiate from a relatively small locus, so that the lesion simulates a many pointed star of which the radii, though often branched, are smooth, as neither intralobular ducts nor alveoli develop along them. Breeding apparently tends to favor their development for only one of the 13 occurred in a nonbreeding animal. Although the change occurs much less frequently than the hyperplastic alveolar lesion, it also is apparently precancerous in nature since Gardner pictures a small adenocarcinoma developing in the center of such an area and, in the present material, this lesion was encountered only in mice of high tumor lines. However, because such areas of hyperplasia are relatively infrequent, from a numerical standpoint they cannot be a very important source of malignant transformation.

The third type of lesion appears to be inflammatory in nature, for although considerable variation exists between the individual nodules included in this group an inflammatory reaction is conspicuous in all. In general, these areas consist of some alveolar hyperplasia, which is, however, usually less pronounced than in the typical precancerous alveolar nodules. About this alveolar center is an inflammatory reaction consisting of a more or less extensive fibroblastic proliferation plus an infiltration of lymphocytes. The extent of the reaction varies widely from nodule to nodule; in some the glandular elements may slightly predominate whereas in others they are seen only with difficulty in whole-mount preparations. However, foci of lymphocytic infiltration without glandular hyperplasia are not to be included with this type of lesion. In most all cases alterations in the epithelial elements are conspicuous. These consist mainly of metaplasia of glandular to squamous-type epithelium, often with a good deal of desquamation of keratinized material into distended alveolar lumina. A few nodules composed entirely of squamous alveoli, without appreciable inflammatory reaction, were encountered, but whether these were the end product of inflammatory nodules could not be determined. It is to be emphasized, however, that the mere presence of a small amount of squamous metaplasia without a concomitant inflammatory reaction does not place a nodule in this inflammatory group. For in breeding mice isolated squamous alveoli appear not infrequently in normal areas of the gland, as well as more rarely in otherwise typical areas of alveolar hyperplasia.

A review of the literature revealed that the inflammatory nodules described above are probably identical with the nodule pictured by Haaland (31, p. 32, Fig. 19), and later described in more detail by Gardner (24). Their relationship to carcinogenesis will be considered later.

A tabulation of the occurrence of these 3 types of lesions in mice of the A and C3H strains, with and without the milk influence, and their reciprocal F_1 hybrids is given in Tables II, III, and IV. When the

Table III

	Age, days	Type of hybrid	No. of glands	Alveolar nodules	Alv. nod. per gland	Duct nodules	Inflammatory nodules
			High Tumor F_1 Hybrids				
Virgin [21]	157	AZ	4	0	0	0	0
	158	ZA	4	6	$1\frac{1}{2}$	0	0
	193	AZ	4	1	$\frac{1}{4}$	0	0
	253 T	AZ	3	6	2	0	0
	259 T	ZA	4	5	$1\frac{1}{4}$	0	0
	264	AZ	4	14	$3\frac{1}{2}$	0	0
	265	ZA	4	8	2	0	0
	265	ZA	4	5	$1\frac{1}{4}$	0	0
	272 T	ZA	4	1	$\frac{1}{4}$	0	1
	278 T	AZ	2	4	2	0	0
	314 T	AZ	4	6	$1\frac{1}{2}$	0	1
	358 T	AZ	2	3	$1\frac{1}{2}$	0	0
	361 T	ZA	3	6	2	0	0
	381 T	AZ	4	12	3	1	0
	435 T	AZ	4	10 (1 CA)	$2\frac{1}{2}$	0	0
	479 T	AZ	4	2	$\frac{1}{2}$	0	0
	485 T *	ZA	4	26	$6\frac{1}{2}$	0	0
	519 T	AZ	4	8	2	0	0
	565 T	ZA	4	14	$3\frac{1}{2}$	0	2
	610 T	ZA	4	38	$9\frac{1}{2}$	0	0
	717	ZA	4	6	$1\frac{1}{2}$	0	0
Breeding [8]	248 T	AZ	2	50	25	0	0
	265 T	AZ	4	32	8	1	3
	267 T	ZA	3	44	$14\frac{2}{3}$	0	0
	272 T	AZ	4	16	4	1	0
	272 T	ZA	4	68 (2 CA)	17	0	1
	376 T	ZA	4	38	$9\frac{1}{2}$	1	2
	383 T	ZA	4	86 (1 CA)	$21\frac{1}{2}$	1	0
	394 T	AZ	4	49	$12\frac{1}{4}$	0	1
			Low Tumor F_1 Hybrids				
Virgin [10]	307	AxZb	4	0	0	0	0
	307	AxZb	4	0	0	0	0
	404	AxZb	4	0	0	0	0
	408	ZbAx	4	0	0	0	0
	420	ZbAx	4	0	0	0	0
	442	ZbAx	4	0	0	0	0
	448	ZbAx	4	0	0	0	0
	448	ZbAx	4	0	0	0	0
	450	AxZb	4	0	0	0	0
	451 *	ZbAx	4	0	0	0	0
Breeding [11]	388	ZbAx	4	0	0	0	2
	388	ZbAx	4	1	$\frac{1}{4}$	0	2
	388	ZbAx	4	(1)	$\frac{1}{4}$	0	2 (1 squam.)
	388	ZbAx	4	0	0	0	1
	415	AxZb	4	0	0	0	1
	415	AxZb	4	0	0	0	0
	415	AxZb	4	0	0	0	0
	415	AxZb	4	0	0	0	0
	494	AxZb	4	0	0	0	0
	498	AxZb	4	0	0	0	0
	509	ZbAx	4	0	0	0	0

Data concerning F_1 hybrid mice included in this histological study. For explanation of notations see legend of Table II. The "squam." in parentheses in "Inflammatory Nodule" column indicates nodule composed entirely of squamous alveoli with no signs of surrounding inflammatory reaction.

frequency with which the alveolar type of nodule occurs in the glands of mice of high tumor lines is compared with that in the corresponding low tumor lines lacking the milk influence, it is obvious that the development of the lesion is dependent upon the presence of this agent, for although alveolar hyperplasias are abundant in the glands of the high-tumor stocks they rarely occur in the glands of the corresponding low-tumor lines. In fact, only 7 nodules were encountered in 197 glands of 51 animals that were genetically susceptible to, and had sufficient hormonal stimulation for, mammary carcinoma but did not develop it because of absence of the milk influence. In 5 of these nodules macrophages were considerably more numerous than in typical alveolar nodules, but as this was the only respect in which they differed from typical precancerous hyperplasias they were included in this group and set off with brackets in the tables. Thus when the milk influence is absent the decrease in the frequency with which areas of alveolar hyperplasia occur is as striking as the decrease in the incidence of mammary carcinoma.

TABLE IV

	Age, days	No. of glands	Alveolar nodules	Alv. nod. per gland	Duct nodules	Inflammatory nodules
			A FEMALES (HIGH TUMOR)			
Virgin [14]	130	4	0	0	0	0
	130	2	0	0	0	0
	248	4	0	0	0	0
	248 *	4	0	0	0	0
	348	4	0	0	0	0
	356	4	0	0	0	0
	358 T	2	0	0	0	0
	452	2	0	0	0	0
	452	4	0	0	0	0
	455	4	0	0	0	0
	455	4	1	$\frac{1}{4}$	0	0
	478	3	0	0	0	0
	478	2	0	0	0	0
	478	4	0	0	0	0
Breeding [9]	233 T	4	6	$1\frac{1}{2}$	0	0
	282 T	4	26	$6\frac{1}{2}$	0	0
	315 T	4	14	$3\frac{1}{2}$	0	0
	323 T	4	40	10	1	1
	345 T	4	54 (3 CA)	$13\frac{1}{2}$	0	0
	354 T	3	11	$3\frac{2}{3}$	0	0
	359 T	4	10	$2\frac{1}{2}$	1	0
	402 T	2	6	3	0	0
	413 T	4	28	7	1	0
			Ax FEMALES (LOW TUMOR)			
Virgin [6]	387	4	0	0	0	0
	387	4	0	0	0	0
	468	4	0	0	0	0
	472	4	0	0	0	0
	477	4	0	0	0	0
	479	4	0	0	0	0
Breeding [12]	257	4	0	0	0	0
	269	4	0	0	0	0
	333	3	0	0	0	0
	333	3	0	0	0	0
	357	4	0	0	0	0
	372	3	0	0	0	0
	438	4	1	$\frac{1}{4}$	0	0
	438	4	0	0	0	0
	438	2	0	0	0	1
	480	4	0	0	0	0
	480	4	0	0	0	0
	504	4	0	0	0	2

Data concerning A and Ax mice included in this study. For explanation of notations see the legend of Table II.

The distribution of these nodules in the various high-tumor lines is also of some interest. Their increase with age, as noted by Haaland (31) and by Gardner, Strong, and Smith (27) is best brought out in the series of virgin Z mice where the number of lesions per gland tends to increase throughout the entire age period studied. Such a tendency is not evident, however, in the virgin hybrid mice after they reach 200 days of age. Also of interest is the fact that although the virgin hybrid mice have an incidence of mammary carcinoma as high as or higher than that of the Z virgins, the number of precancerous lesions in their glands is noticeably less. This would indicate that although a close correlation exists between these hyperplastic areas and cancer, the tumor incidence of a strain may not be strictly paralleled by the number of precancerous lesions developing in the mammae. That breeding increases the number of nodules and brings about their earlier development, as is also the case for tumors, is brought out. In all high tumor strains the nodules are much more frequent in breeding animals than in virgins of corresponding age. The earlier appearance is emphasized when the glands of the Z mice 130 to 135 days of age are compared; here only 2 small nodules were found in 12 glands of 3 virgin mice, whereas 30 were present in 8 glands of 2 mice that had had but 1 litter each.

The distribution of the inflammatory nodules, on the other hand, is quite different from that of the alveolar type of lesion. It is at once evident that this type of nodule occurs much less frequently than does the typically precancerous alveolar hyperplasia; similarly, though, they appear to be more common in breeding than in virgin animals, and in the C3H strain than in either the A strain or hybrids between the two. It can be seen, however, that there is no significant difference in the frequency with which they appear in the glands of the high and low tumor lines. On the basis of this observation, and from the fact that no evidence could be found suggesting the development of carcinomas within them, it is felt that this inflammatory type of nodule does not predispose to the usual spontaneous carcinoma of mice.

Virgin mice of the A strain have a low incidence of spontaneous mammary carcinoma, for although they possess both milk influence and genetic susceptibility, they apparently lack the correct hormonal stimulation for tumor production. Like the glands of low tumor mice that lack only the milk influence, the mammae of virgin A mice only rarely possess hyperplastic alveolar nodules, for in the present material only 1 small alveolar area was encountered in 41 glands from 12 mice that were 248 days of age or older; and no nodules were found in 2 glands from the 1 that had an undifferentiated adenocarcinoma of the mammary gland at the time of autopsy. This is in sharp contrast to the frequent occurrence of alveolar nodules in the glands of multiparous mice of the A strain, in which the hormonal stimulation has been altered by breeding so as to be adequate for the production of cancer in a high percentage of individuals. From these observations it would appear that the hormonal stimulation to which the mammae are subjected is as important as the milk influence for the development of precancerous alveolar hyperplasias.

In order to complete the picture an attempt was made to determine the role played by genetic susceptibility in the development of precancerous lesions. This third factor, however, is less precise than the preceding two, for few strains of mice, if any, are completely resistant to the development of mammary cancer when the milk influence and a sufficient hormonal stimulation are supplied. Thus a review of the literature reveals that when mice of the nonsusceptible C57 black strain have been given the milk influence and either normally or forced bred, the incidence of tumors has varied from 9.1 to 76 per cent (2, 3, 5, 8, 12, 30). However, in lieu of a more resistant strain, a line of C57 black mice was chosen for this experiment, 10 females, to be designated Ba_1, being foster nursed by strain A females. They were then intensively bred, and the young were allowed to suckle their own mothers. The Ba_2 females so produced were then forced bred, in that they were continuously housed with males and the young were removed shortly after birth. All animals of both the Ba_1 and Ba_2 generations that did not have 3 or more litters were excluded from the experiment. Since an earlier investigation indicated that the milk influence was passed through at least 1 generation of C57 black mice (12), it was assumed that the mice of the Ba_2 generation possessed this agent.[2] However, despite the addition of these 2 factors only 10.3 per cent of 58 mice developed mammary carcinoma, indicating a rather high degree of genetic nonsusceptibility. The glands of 2 tumorous and 4 nontumorous animals were prepared for histological study. Although the number of cases is small, the results are interesting, for in the glands of the mice developing cancer areas of alveolar hyperplasia were numerous and 2 ductular nodules were found; on the other hand, only 1 small alveolar nodule was encountered in the glands of the nontumorous mice. These data would indicate, then, that the genetic constitution of the animal with regard to susceptibility to the development of mammary can-

[2] In an article published since the completion of this portion of the experiment doubt is cast upon passage of the milk influence through C57 black mice (4). Pertinent material concerning this point will be considered in some detail in our Discussion.

cer also affects the development of hyperplastic alveolar lesions in the breast.

To summarize the findings on the development of precancerous alveolar hyperplasia in the mice studied here, it may be said that this lesion occurred frequently in the glands of all mice belonging to high tumor lines or, in the C57 black group, in those developing cancer; but only very infrequently in mice of the low-tumor lines, no matter which of the 3 principal factors was lacking so as to yield this low incidence. Thus it would appear that all the 3 factors primarily responsible for the development of mammary cancer in mice are also necessary for the development of these alveolar hyperplasias.

DISCUSSION

The increase of lateral budding with the presence of the milk influence, as reported by van Gulik and Korteweg (42) and confirmed by Shimkin, Grady, and Andervont (37), was certainly not discernible in the material presented here or in that of a preliminary experiment carried out in this laboratory and outlined in the Introduction. Intralobular duct development was found to vary from strain to strain and with age in virgin mice of the Z and Zb lines, but when these factors were taken into account no difference in lateral budding in the glands of either virgin or breeding mice could be associated with the presence of the milk influence.

The material presented here differs in two respects from that reported by the other authors. In the first place, different strains of mice were used so that conceivably, though not probably, the variance might be entirely the result of strain differences. The second dissimilarity is that the foster nursed animals studied by the other investigators were of the first fostered generation, whereas here the lines lacking the milk influence had been foster nursed several generations previously, with all subsequent generations suckling their own mothers. By what mechanism this difference in procedure could affect the definitive structure of the mammary glands is not evident at the moment. However, in view of the lack of correlation observed in the present experiment and the absence of a demonstrable alteration following injection of the milk influence into young, low tumor, ABC mice as indicated by the preliminary study, it seems fair to conclude that the presence of the milk influence *per se* does not effect the development of lateral buds.

The degree of lateral budding in the glands of virgin mice may, however, be related to the hormonal constitution of the animal, and more particularly to the inherited hormonal influence (16, 32, 18). Although only 2 strains of mice were employed here, by utilizing the published material of other investigators a somewhat larger number of inbred strains may be considered (40, 42, 43). Thus 2 strains, the C3H, or Z, and the dba, develop a high incidence of mammary cancer when kept as virgins, and therefore possess the inherited hormonal influence. In the glands of both of these strains lateral buds are abundant. On the other hand, in the glands of mice of the A and C57 black strains, in which this inherited hormonal factor is lacking, the ducts are smooth, with intralobular ducts protruding from the walls only infrequently.[3] Although mice from too few inbred strains have been studied as yet to warrant a definitive conclusion, the suggestion is that the lateral budding is more extensive in virgin mice of strains possessing the inherited hormonal influence and, in this way, is related to the development of cancer in virgin mice.

The relationship of the nodular areas of alveolar hyperplasia to carcinogenesis is, however, more direct in nature and more firmly established. Three distinct lines of evidence have been presented by other investigators to indicate their precancerous nature; they occur much more frequently in high than in low tumor strains of mice, histological transitions can be found between them and frank carcinoma, and a certain per cent grow progressively when transplanted, forming true cancers (21, 24, 27, 31, 40, 42). The first 2 of these are further emphasized by the present investigation, and data regarding the etiologic factors necessary for their development are presented.

Although these lesions have been produced in mice by the injection of estrogenic hormones and the etiologic importance of these substances stressed (25, 29, 44), Gardner, Strong, and Smith (27) stated that ". . . . the development of the small hyperplastic nodules may be considered to be due to factors passed from parent to offspring in association with other factors predisposing to cancer." Not only is this concept borne out by the present investigation, but it is also made evident that all 3 of the primary factors responsible for tumor development must be present in order for these nodules to develop with any frequency. That the milk influence is necessary is reflected in the glands of the Zb and Ax mice and their hybrids. The dependency of this lesion upon a quantitatively and/or qualitatively adequate hormonal stimulation is evidenced in the glands of the virgin A strain mice. And the effect of the lack of a genetic susceptibility is seen in the noncancerous, foster nursed, C57 black animals. All 3 of these groups have a low incidence of

[3] Mice of the C57 black strain may be included here, for if males of this strain are mated with females of the A strain the F_1 virgin hybrids have a low incidence of mammary cancer (10). Since the inherited hormonal influence is transmitted as a dominant character, this would indicate that the factor is not present in the C57 black strain.

mammary carcinoma because each lacks a single, though different, "primary" factor, and in each instance alveolar hyperplasias occur but rarely.

Further evidence of the importance of hormonal stimulation to the development of the alveolar nodules is found in glands of C3H mice that have been chronically underfed. At the time that such data were originally presented (35), the glands of too few animals had been studied to make a comparison of the frequency with which precancerous nodules appeared of significance. Since then, however, additional glands have been prepared, and it has become evident that the number of nodules in the calorically restricted, and thus nontumorous, mice is much reduced. Less than 1 nodule per 4 glands, *i.e.*, glands 2 and 3 bilaterally, has been found in these mice after 340 days of age. When this incidence is compared with the frequency of the lesion in normally fed mice of a comparable age (Table II) the difference is striking. It is interesting to note, nevertheless, that precancerous nodules are more frequent in the calorically restricted C3H mice, in which no tumors have occurred in 43 animals exceeding 300 days of age so far observed, than in virgin A mice, approximately 4 per cent of which develop mammary cancer. This may be of etiological significance with regard to hormonal stimulation. Of the 2 groups of glands those of the calorically restricted C3H mice apparently would be subjected to less estrogenic stimulation, for these mice had been so severely underfed that they were in an almost constant state of anestrus, while normal virgin A mice show regular estrous cycles. Although such comparisons cannot be carried too far, as different strains of mice are involved, these observations suggest that the estrogenic hormones may not be the only one of importance in the development of this lesion. Further investigation into this matter is under way at the present time.

Interpretation of the findings in the glands of the foster nursed, forced bred, C57 black mice is complicated not only by the incompletely understood and therefore vague nature of genetic nonsusceptibility, as mentioned before, but also by an apparent confusion within this strain of mice as maintained in different laboratories. Rather extensive evidence has been presented to indicate that widely different sublines exist in this strain (16); this is evidenced by the wide variation in the incidence of cancer, ranging from 9.1 to 76 per cent (2, 3, 5, 8, 12, 30), noted by different authors when these animals have been supplied with the milk influence and allowed to breed. Of more importance from the standpoint of the present paper, however, is the possibility that these different sublines transmit the milk influence to a very different degree. Earlier data indicated that C57 black mice, only 10.6 per cent of which developed mammary cancer when given the milk influence, transferred sufficient amounts of this agent to nursing young for a high percentage of genetically susceptible test animals to develop cancer when nursed by C57 black foster mothers (12). Haagensen (30) found that in his subline 76 per cent of the breeding females foster nursed by high tumor RIII females developed cancer, and that a similar percentage of the young born to and nursed by these fostered C57 black females also developed cancer, indicating that the milk influence had been transferred by these "high tumor" black animals.

On the other side of the picture Heston, Deringer, and Andervont (33), in an article published since the completion of our experiment, present evidence that hybrid mice back-crossed to their C57 black line transmit the milk influence less well than do similar hybrids back-crossed to their C3H strain. Further, Andervont (4) found that 2 females of his black strain, though nursed by C3H foster mothers, did not transfer the milk influence to genetically susceptible young nursed by them.

Analyzing our own data by generation we found that whereas 40 per cent of the 10 foster nursed C57 black females developed cancer, only 4.2 per cent of 48 offspring nursed by them did so. Although the number of animals is small, the observations fall more in line with those of Heston and Andervont.

It seems obvious from these data that no general conclusions concerning the passage of the milk influence by C57 black mice can be reached at present, and that further investigation is necessary, keeping in mind that definite sublines of this strain of mice do exist. An analysis of the 4 noncancerous C57 black mice used for histological study in the present experiment indicates, however, that at least 3 of them probably did possess the milk influence. For 1 was of the Ba_1 generation 1 of the Ba_2 animals had been nursed by a cancerous mother, and a second had a cancerous sister. It is of further interest that the 1 nodule that occurred in this group of noncancerous mice was in a Ba_2 mouse that had neither a cancerous mother nor any cancerous sisters. It would seem, therefore, that although the observations on these few mice cannot be considered conclusive, they do appear to be significant.

The inflammatory type of nodule deserves some comment here, for although it has been pictured by Haaland (31) and described by Gardner (24), no information has been presented previously to distinguish it from the precancerous alveolar hyperplasia. It seems rather certain from the present observations, however, that this inflammatory lesion is not precancerous in nature, so far as the usual spontaneous disease in mice is concerned. It was found to occur with equal frequency in the glands of low and high tumor strains,

and in spite of its rather frequent occurrence in older multiparous mice of the Zb and low tumor hybrid lines no mammary tumors have been observed in 237 of these mice in the last 3 years. Thus on the basis of histology and carcinogenic potentialities, the inflammatory nodule seems to be quite distinct from the precancerous alveolar hyperplasia.

Gibson (28) described lesions in the mammae of a high tumor strain of mice that appear to be somewhat similar to the inflammatory type, in that they consist of dilated alveoli surrounded by a rather extensive lymphocytic infiltration. She felt that carcinomas developed in such areas, but it cannot be ascertained from her photographs whether or not these areas of "chronic cystic mastitis" are the same as the inflammatory nodules described here.

The present material throws but little light upon either the mode of development or the factors important in the etiology of this inflammatory nodule. It is evident, however, that pregnancy and/or lactation favor, though are not necessary for, its development. But whether it results from a reaction about stagnant secretion, or whether the squamous metaplasia of glandular epithelium, possibly as a response to hormonal stimulation, is the initial alteration, could not be determined. It does seem probable, however, that it differs from the precancerous lesions from its inception, as no convincing evidence could be found to indicate transitions between the two types.

SUMMARY

A histological study was made to correlate the architecture of the mouse mammary gland and the 3 "primary" factors required for spontaneous mammary carcinogenesis: an inherited susceptibility, quantitatively and/or qualitatively adequate hormonal stimulation, and the milk influence. For this investigation several low tumor lines of mice, each lacking a single, though different, "primary" factor, were selected and compared with suitable mice that possessed all 3 of these factors and thus had a high incidence of mammary cancer. In this way the effects of a lack of each factor could be determined individually, and the following points were established:

1. The presence of the milk influence *per se* does not alter the extent to which lateral buds occur along the larger ducts of the mammary gland.

2. In the present material, as well as in that previously reported by other authors, lateral budding is more extensive in virgin mice of strains that possess the inherited hormonal influence than in those that lack this factor.

3. Precancerous nodules of alveolar hyperplasia occur frequently only in mice of high tumor groups and are very uncommon in those of low tumor lines, irrespective of which one of the "primary" factors is lacking. From this it is concluded that the same 3 factors that are etiologically important for the development of mammary cancer are necessary for the development of precancerous alveolar hyperplasias.

4. Areas composed of an overgrowth of fine ducts were encountered in the mammae of mice belonging to high tumor lines. These, in all probability, are also precancerous in nature, but because they occur with relative infrequency they cannot represent a very common source of malignant transformation.

5. Inflammatory nodules, consisting of some alveolar hyperplasia usually exhibiting squamous metaplasia of the glandular epithelium and a surrounding inflammatory reaction, did not appear to be precancerous in nature. These occur with equal frequency in low and high tumor strains, and no transitions between them and frank carcinoma could be demonstrated. Etiologic factors important for the development of this type of lesion could not be determined completely, but pregnancy and/or lactation were found to favor their development.

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Methylcholanthrene Squamous Cell Carcinoma of the Rat Prostate with Skeletal Metastases, and Failure of the Rat Liver to Respond to the Same Carcinogen

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Moore (3) recently stated that adenocarcinoma of the prostate similar to that observed in man had not been produced in experimental animals, citing the earlier experiments of Moore and Melchionna (4), who observed squamous cell cancer and sarcoma in the rat prostate following the injection of benzpyrene. They observed the columnar epithelium undergoing squamous metaplasia in contact with, or in proximity to, the benzpyrene cysts, and induced cancer in both normal and atrophic prostates. No metastases were observed from any of these induced neoplasms.

The object of the present paper is to report an induced squamous cell carcinoma of the prostate in a rat with a history similar to that frequently observed in cases of adenocarcinoma of the human prostate. Skeletal metastasis occurred in the primary host and in hosts bearing subcutaneous transplanted growths of this tumor.

MATERIAL

Pellets of compressed methylcholanthrene crystals weighing approximately 3 mgm. were implanted in the prostate of 6 month old rats of two inbred lines: albino Fischer, line 344; and black agouti Irish, A × C line 9935. The surgical openings were closed with one or two interrupted silk sutures. Coincidentally, similar pellets were placed in previously prepared pockets, deep in the parenchyma of the livers of the litter sisters of these males.

RESULTS

The results are summarized in Table I. In an average of 329 days 10 of the 17 A × C male rats developed squamous cell carcinoma of the prostate, and one Fischer male developed a tumor at this site. No growths of the liver were observed in either line. Fig. 1 shows the life span and tumor history of the individual rats. The earliest neoplasm was discovered after 198 days, and all the A × C males that survived for 400 days developed prostatic tumors. All the Fischer line 344 males died in less than 400 days, but of the 2 that survived for 333 days 1 had a tumor and 1 survived this period by a few days without developing a neoplasm. On the other hand, 7 of the line 9935 rats with pellets implanted in the liver survived for a period considerably longer than any of those with pellets in the prostate without developing liver tumors. These negative results are in keeping with those previously reported for intrahepatic injection of dibenzanthracene and benzpyrene by Ilfeld (2); Oberling, Sannié, and Guérin (5); Woglom (8); and Rusch, Baumann, and Maison (6), also with our own unreported experience following the intrahepatic injection of benzpyrene in paraffin. In the latter case the

TABLE I: THE NUMBER AND PERCENTAGE OF INDUCED CANCERS IN RATS IMPLANTED WITH METHYLCHOLANTHRENE PELLETS, AND SURVIVING FOR AT LEAST 200 DAYS THEREAFTER

Strain	Sex	No. of rats	Dose of M-C, mgm.	Site implanted	Average age implanted, days	Average days survived after implantation	Induced cancers No.	Induced cancers Per cent
A × C	female	15	3	liver	185	528	0	0
Fischer	female	9	3	"	200	257	0	0
A × C	male	17	3	prostate	186	329	10	59
Fischer	male	9	3	"	188	241	1	17

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nodules of paraffin carrying the carcinogen tended to work to the surface of the liver, and since the capsule was very thin the majority healed, leaving no scar in the liver and the nodule of paraffin free in the peritoneal cavity. This was not the case in the liver implantations reported here. At the postmortem examination the pellets were found *in situ,* surrounded by a dense fibrous capsule (Fig. 2). The bile ducts persisted in the capsule for a considerable time (Fig. 3). Proliferating bile ducts were found in the capsule of a rat that died 524 days after implantation of the pellets (Fig. 4), and dilated and cystic ducts (Fig. 5) in the capsule of the methylcholanthrene pellet 679 days after implantation. Two rats outlived the latter, but no neoplasms developed. Rats of these two lines, 344 and 9935, had the highest incidence of *Cysticercus* sarcomas of the liver (1), so there can be no question of the susceptibility to neoplasia of the hepatic stroma. Two of the A×C line 9935 females with liver pellets developed spontaneous neoplasms, 1 an early sarcoma

TABLE II: HISTORY OF M-C TUMOR 951 AFTER TRANSPLANTATION

Tumor gen.	No. of rats	Sex	Age inoc., days	Days to death	Number positive	Average diameter of tumor, cm.	Location of metastases: Lungs	Lymph node	Skeleton
1	10	male	107	105	8	1.8	0	0	0
2	6	"	179	164	4	2.1	1	1	0
2	6	female	124	54	1	1.8	0	0	0
3	12	male	285	82	8	2.3	0	3	0
3	10	female	187	199	7	2.9	0	7	1
4	6	male	194	152	3	3.2	1	3	0
5	4	"	467	70	3	3.8	0	0	0
5	2	female	560	104	1	2.2	0	1	0
6	10	male	167	54	10	3.1	1	2	0
7	17	"	77	98	15	4.1	15	15	10
7	20	female	124	94	19	3.7	16	18	15
8	7	male	130	46	2	2.8	1	2	0
8	10	female	169	70	6	2.4	6	6	5

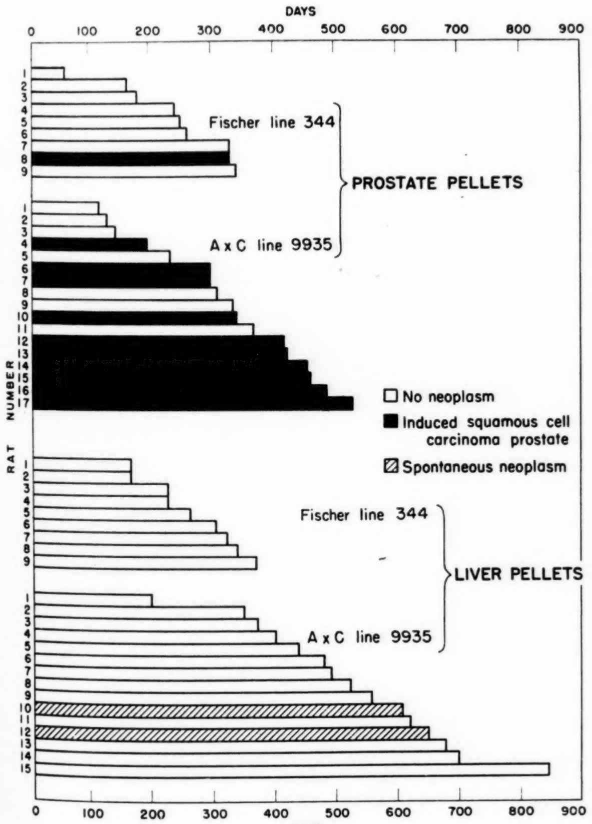


FIG. 1.—Length of survival and tumor history of rats implanted in liver and prostate with methylcholanthrene pellets.

of the lungs and the other an adenoma of the mammary gland. Both tumors were unrelated to the experimental procedure.

In most instances the appearance of the prostatic carcinoma was associated with considerable inflammatory reaction and abscess formation. Fig. 6 shows a section through the prostate with some of the methylcholanthrene crystals *in situ,* 166 days after implantation, while Fig. 7 illustrates the more typical chronic prostatitis and squamous metaplasia that preceded the appearance of the tumors.

The 11 induced neoplasms were all squamous cell carcinomas. Fig. 8 reproduces a section through one of these tumors, M-C 951, obtained from a biopsy after it had become palpable, and Fig. 9 shows a section through a metastasis invading the wall of the stomach, which was obtained from the postmortem specimen 2 days later. The postmortem examination also revealed extensive dissemination of the tumor to the mesentery, omentum, liver, adrenal, colon, diaphragm, and ribs. Biopsy specimens were obtained from 2 of the tumors, and in each case a few rats were inoculated with grafts. Both fragments grew, but one tumor was discarded after the first generation, when postmortem examination of the primary host of the other revealed skeletal metastases.

Table II shows the transplantation history of tumor M-C 951 for 8 generations. In spite of the fact that

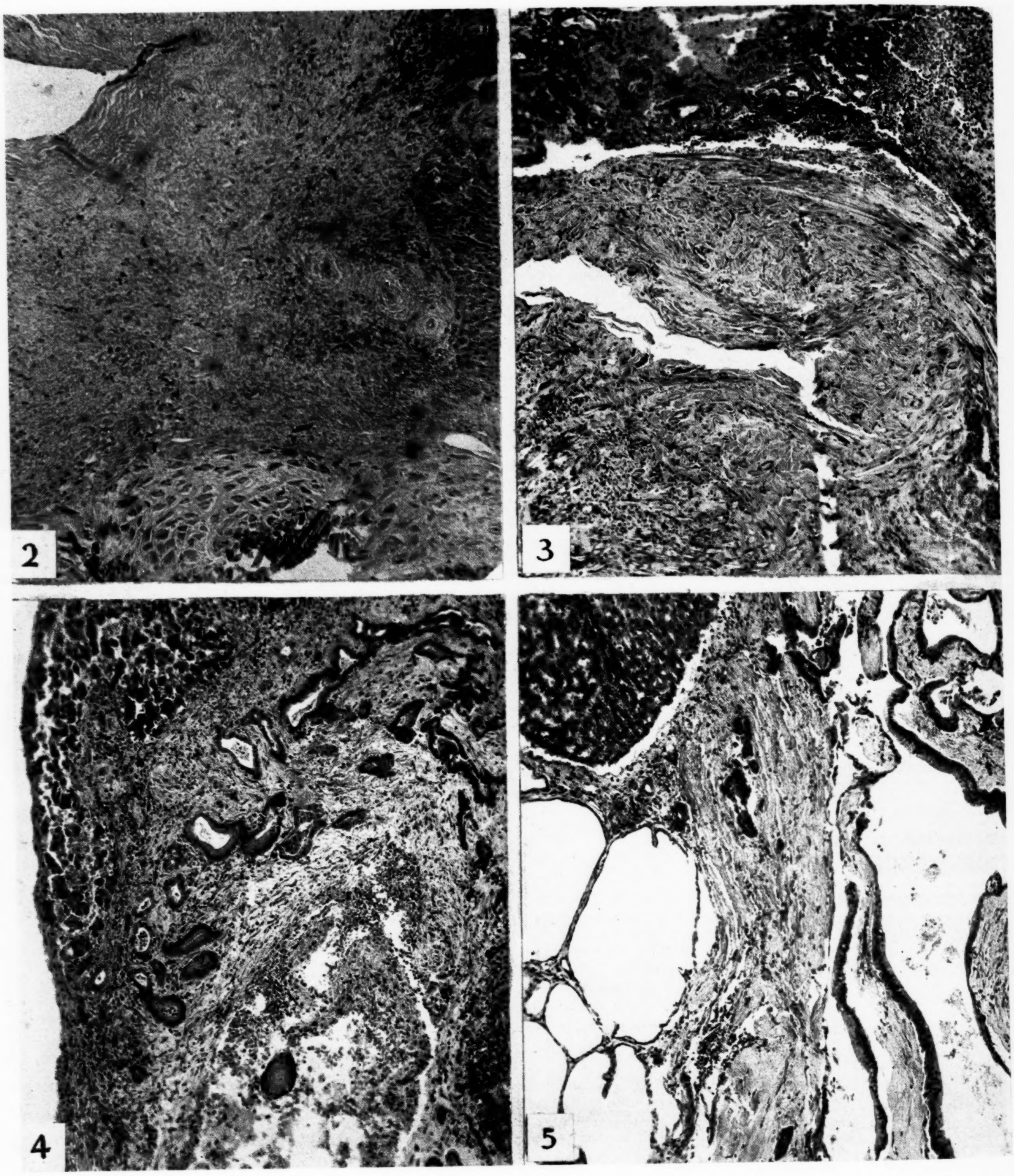
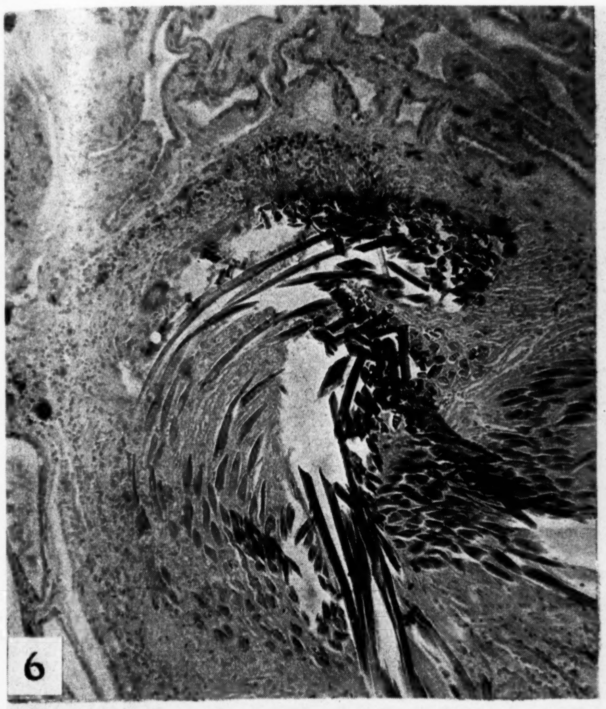
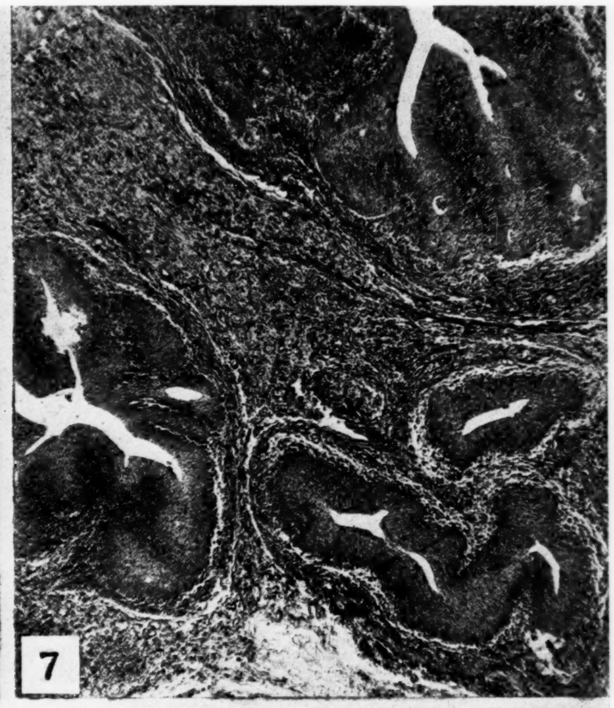
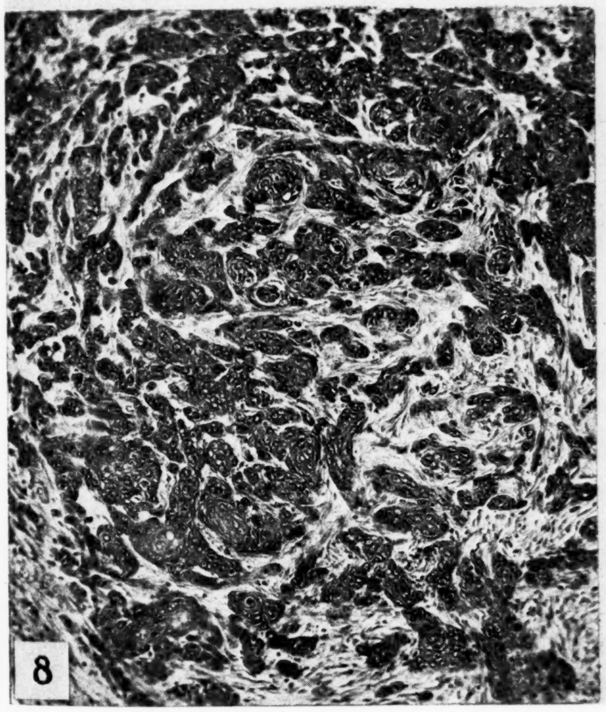


FIG. 2.—Dense fibrous capsule around methylcholanthrene pellet in liver of rat 167 days after implantation. Mag. × 125.
FIG. 3.—Persistence of bile ducts in capsule of methylcholanthrene pellet in rat's liver after 232 days. Mag. × 125.
FIG. 4.—Proliferating bile ducts in capsule of methylcholanthrene pellet 524 days after implantation. Mag. × 125.
FIG. 5.—Dilated and cystic ducts in capsule of methylcholanthrene pellet 679 days after implantation. Mag. × 125.







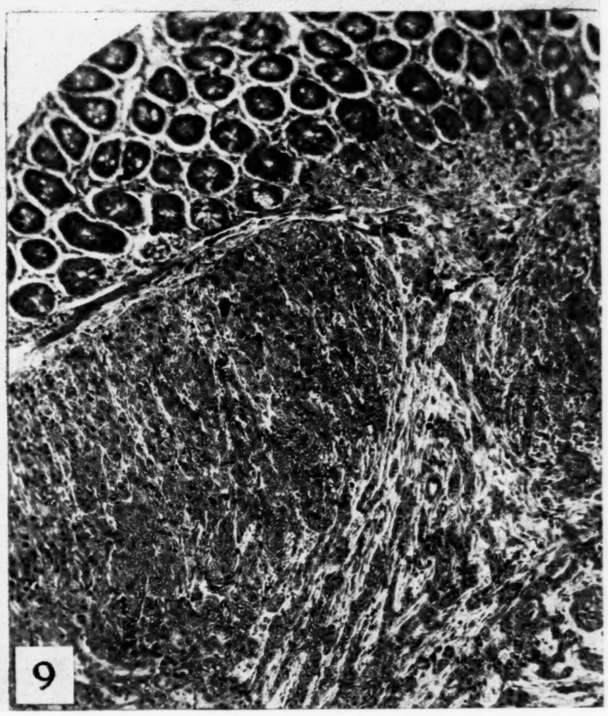
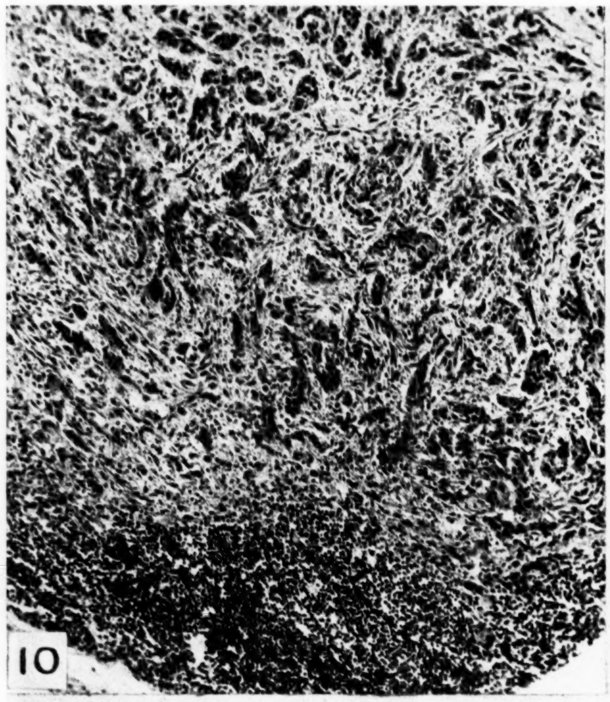


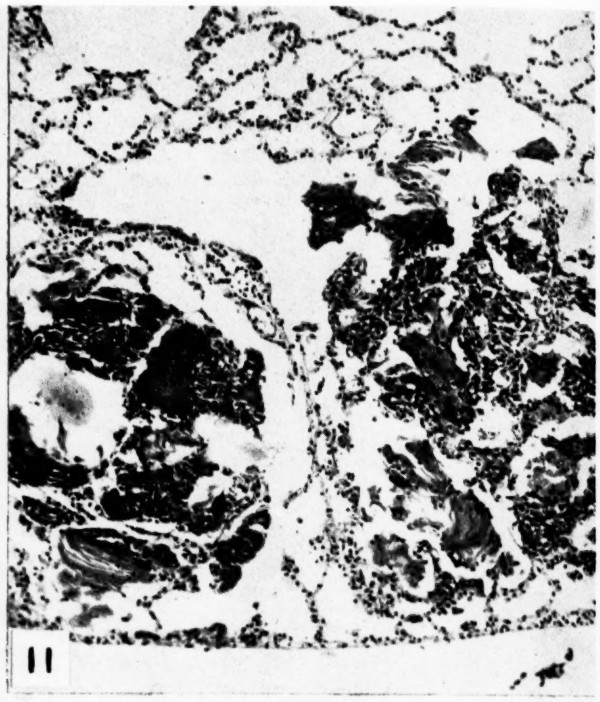
FIG. 6.—Methylcholanthrene crystals in rat prostate, showing some connective tissue reaction 166 days after implantation. Mag. $\times$ 125.

FIG. 7.—Chronic prostatitis with squamous metaplasia 251 days after implantation of methylcholanthrene pellet. Mag. $\times$ 50.

FIG. 8.—Tumor M-C 951. Squamous cell carcinoma of prostate 300 days after implantation of methylcholanthrene pellet. Mag. $\times$ 125.

FIG. 9.—Metastasis of M-C 951 invading wall of stomach. Mag. $\times$ 125.





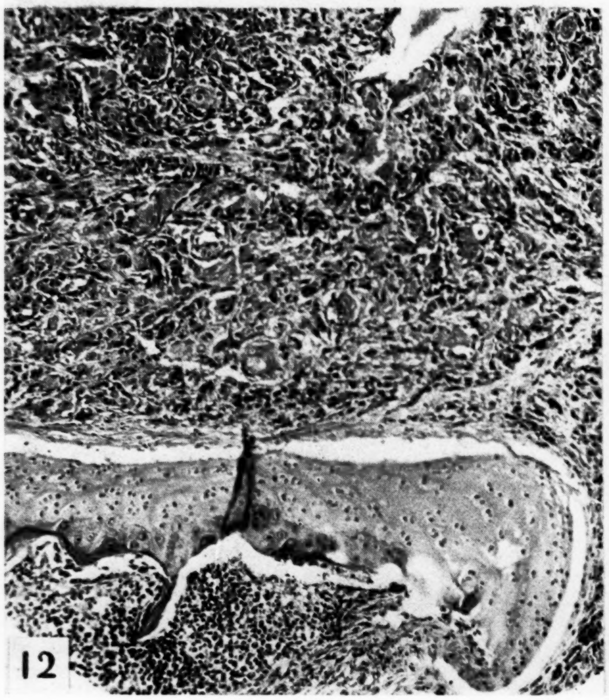


Fig. 10.—Lymph node metastasis of M-C 951/7A. Mag. × 125.

Fig. 11.—Lung metastasis of M-C 951/7A. Mag. × 125.

Fig. 12.—Skeletal metastasis of M-C 951/7A. Mag. × 125.

Fig. 13.—$\frac{\text{R } 2426}{\text{25 C}}$, a mammary adenocarcinoma, transplanted in prostate. Mag. × 50.

the primary host was from the 24th brother-by-sister generation of line 9935, the transplanted tumors grew progressively in only about 70 per cent of inoculated rats of the same line. It grew equally well in males and females, as shown in Table II. Whether the failures were due to the quality of the inoculum or to heterozygosity of the stock is not known. The transplanted tumors ulcerated fairly early, and for several transfers the tissue for inoculation was obtained from a lymph node metastasis in a rat with an ulcerating growth. The history in these cases, however, was no different from that of the earlier transfers. No systematic search for skeletal metastases was made until the seventh generation, and these tabulations are based on gross findings confirmed by microscopic examination. Skeletal metastases were found incidentally in 1 rat of the third generation that had a relatively slow-growing tumor and a longer than average survival period. Lymph node metastases had been noted in several rats previously, and 1 in the second generation had shown lung metastases. In the seventh and eighth generations the tumor rats were allowed to die; postmortem examination included a complete inspection of the skeleton, lungs, and lymph nodes, and positive material was preserved for microscopic confirmation. In the seventh generation (Table II) all the males and most of the females had lung and lymph node metastases, and 66 per cent of the males and nearly 80 per cent of the females had skeletal metastases. Figs. 10, 11, and 12 are representative sections respectively of the lymph node, lung, and skeletal metastases in these rats. The ribs, sternum, and vertebrae were the usual sites of skeletal involvement (Fig. 14). These findings indicate that something inherent in the cells of the primary tumor, rather than its location or morphology, determined the propensity of the neoplasm to form skeletal metastases. Fragments of this tumor, which was presumably derived from metaplastic squamous epithelium in the prostate, growing in the subcutaneous tissues of normal rats of either sex, consistently formed metastases in the bones, a unique site in our experience with transplanted tumors in the rat.

Wallbach (7) obtained metastases in distant organs by implanting the Ehrlich carcinoma in the kidney, ovary, spleen, and liver. As a partial test of the location of the growing neoplasm as a determinant of skeletal metastases, a few rats were implanted in the prostate with grafts of $\frac{\text{R } 2426}{25\text{ C}}$, a transplantable mammary adenocarcinoma. The tumors grew progressively (Fig. 13) and were followed by weekly roentgenograms for possible skeletal involvement. All were negative. The controls for this experiment (Table III) included 5 normal males with axillary implantations, 6 castrated females, and 4 females treated with 5 mgm. of diethylstilbestrol. A careful postmortem examination revealed no metastases in the lungs, skeleton, or superficial lymph nodes of these rats with transplanted tumors in the prostate, although they outlived the controls with axillary tumors by an average of at least 10 days. Gross lung metastases were observed in only 1 of the controls.

TABLE III: HISTORY OF TRANSPLANTED TUMOR R 2426/25C

Group	Location	No. of rats	Age inoc., days	Days to death	Average diameter of tumor, cm.	Location of metastases: Lungs	Lymph node	Skeleton
Intact males	axilla	5	61	97	3.1	0	0	0
Intact "	prostate	5	65	113	4.0	0	0	0
Castrated females	axilla	6	67	97	3.1	1	0	0
Females with 5 mgm. stilbestrol	"	4	68	103	3.5	0	0	0

FIG. 14.—Skeletal metastases in rat with seventh subcutaneous transplantation of M-C 951.

SUMMARY

1. Squamous cell carcinomas of the prostate were induced by 3 mgm. pellets of compressed methyl-

cholanthrene crystals in 1 of 9 Fischer line 344 and 10 of 17 A×C line 9935 rats that survived an average of 241 and 329 days respectively after the pellets were implanted. One of these tumors had produced generalized peritoneal and skeletal metastases.

2. The litter sisters of these males, 9 Fischer line 344 and 15 A×C line 9935 females, survived an average of 257 and 528 days respectively after 3 mgm. methylcholanthrene pellets were implanted in the liver, but failed to develop any hepatic neoplasms.

3. One of the induced prostatic carcinomas was successfully transplanted to the subcutaneous tissues, where it regularly metastasized to the lungs, superficial lymph nodes, and skeleton.

4. Five successful transplants in the prostate of a mammary adenocarcinoma, $\frac{\text{R } 2426}{\text{25 C}}$, were followed by weekly x-ray examinations but no skeletal metastases were observed. Hence location of the induced tumors in the prostate was not responsible for their dissemination.

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A Comparative Study of the Ovaries of Virgin Mice of the dba and C57 Black Strains*

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It has been reported by several investigators that the mammary glands of female mice from strains varying in susceptibility to spontaneous mammary carcinomas show characteristic differences (2, 3, 5, 8). Since the internal secretions of the ovaries influence the development of the mammary glands, the question arises whether differences exist in the ovaries of the various strains. To determine this a comparative study of the ovaries of virgin female mice of the dba high-mammary and the C57 black low-mammary gland tumor strains has been conducted.

MATERIAL AND METHOD

All animals used were virgin females of the highly inbred dba and C57 black strains. They were killed at monthly intervals, and at the ages where the greatest differences occurred, several mice of each strain were used. Fixation and staining methods were uniform. Serial sections were made of the right ovaries and oviducts of 52 dba and 52 C57 black mice. The following observations were made.

1. If ova were present in the oviducts, their number and position were noted.
2. A value proportional to the approximate total volume of each ovary was determined by multiplying the three largest perpendicular diameters. Two of these were obtained by measuring the two greatest diameters of the largest of the serial sections, the third by multiplying the number of sections into which an ovary was cut by the thickness of each section. These values were determined in millimeters.
3. In the serial sections of each ovary the total number of corpora lutea was determined, and to insure accurate counts where many were present every fifth section was projected and drawn on a sheet of paper and each corpus was identified.
4. Whenever ova were present in the oviducts the newest set of corpora lutea could always be differentiated from the older sets. The number of the newest set was determined, but no attempt was made to separate the older ones into age groups.
5. The presence of hyaline areas occurring in the corpora lutea of older dba mice was noted.
6. Observations were made on the presence of yellow lipochrome cells in the ovarian stroma.
7. The occurrence of ovarian cysts was noted.

OBSERVATIONS

The observations are presented in Tables I and II, and as they are self-explanatory only some general remarks will be made.

Atresia.—The ovaries of the 1 and 2 month old animals of both strains contained many follicles in various stages of atresia. This is a normal occurrence that, according to Kingery (6), is most noticable between the ages of 25 and 40 days. Different phases of follicular atresia were present in the ovaries of the older mice also, though it did not occur so frequently. As Engle (1) has made a careful quantitative study of follicular atresia in the mouse no attempt was made to include such an examination in this investigation.

Corpora lutea atretica were found frequently in the ovaries of mice of both strains. These bodies developed from medium or large follicles, from which the ova were not liberated, although the follicular cells around them changed into lutein cells. In some of the large follicles the lutein cells did not fill the follicles completely and central cavities, often filled with blood, were present. In the medium-sized follicles that had changed into corpora lutea atretica the centrally located cells had often accumulated yellow lipochrome in their cytoplasm (Fig. 1).

Lipochrome cell arrangement.—As the atretic corpora lutea shrank and gradually lost their identity the yellow lipochrome-containing cells in both strains became incorporated in the ovarian stroma; however, the manner of this incorporation differed between the two strains in the following ways.

In the ovaries of the dba mice the lipochrome cells diminished in size and became dispersed; they were present in the ovaries of all mice over 4 months of age. In the ovaries of the C57 black mice these cells hypertrophied and remained clustered, forming nodules of

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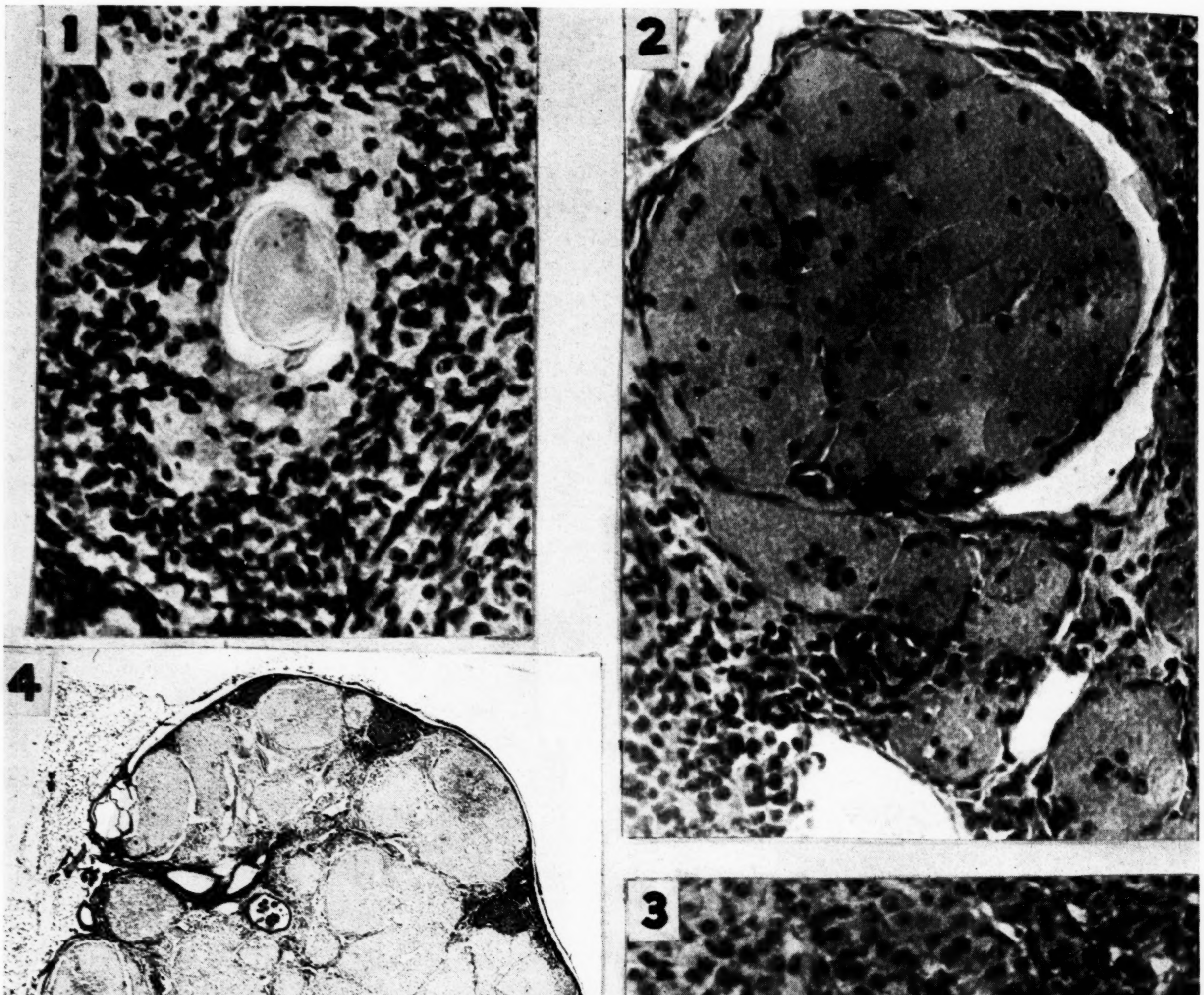
TABLE I: OBSERVATIONS ON THE OVIDUCTS AND OVARIES OF DBA MICE

			Ovary			
			Corpora lutea			
Mouse number	Age. months	Ova in oviduct, total number	Number of newly formed	Total number	Approximate volume, cu. mm.	Cyst
969	1				0.85	
2377	2				2.97	
975	2				2.80	
3094	3			10	5.90	
1032	3			6	3.00	
3120	4			11	5.80	
1065	4	4	6	14	3.72	+
2397	5			11	10.97	
2998	5	5	5	11	4.76	
2433	6	5	5	17	7.12	+
2976	6			28	10.39	
3042	7			11	6.67	
1142	7	6	6	28	10.23	
2971	8	5	5	20	14.47	
2459	8			*	15.92	
1193	8	5	5	13	6.04	
2999	9			16	14.22	
2410	9			29	13.39	
1221	9	5	5	21	11.78	
3123	10	5	5	27	12.01	
1298	10			25	11.68	
2967	11			20	10.83	
2966	11			7	8.00	+
1327	11			35	11.17	
2969	12	6	6	21	9.36	
2968	12			8	8.50	+
1360	12	3	7	22	12.67	
2941	13			8	4.44	
2389	13	8	8	30	16.49	
1412	13	5	5	27	14.61	
2972	14			8	4.84	+
2392	14	6	6	22	17.64	+
959	14			7	6.99	+
2391	15			21	9.66	+
2390	15	6	6	11	8.82	
991	15			*	25.96	
2460	16	3	5	18	7.80	+
1460	16			23	9.72	
1008	16			19	6.66	
3082	17	6	6	15	13.00	
2427	17			12	7.80	
3125	18	5	5	15	6.42	
1745	18			18	12.82	+
2533	19	5	5	5 †	15.84	+
1630	19			18	9.90	
1145	20			10	4.64	
1673	20			4 †	4.19	+
1692	21			‡	9.36	+
1726	22			‡	6.12	+
1934	23			‡	7.56	
1954	24			3	4.68	+
2066	25			‡	7.78	+

* Completely luteinized.
† Partially luteinized.
‡ Completely hyalinized.

TABLE II: OBSERVATIONS ON THE OVIDUCTS AND OVARIES OF C57 BLACK MICE

			Ovary			
			Corpora lutea			
Mouse number	Age, months	Ova in oviduct, total number	Number of newly formed	Total number	Approximate volume, cu. mm.	Cyst
957	1				0.93	
995	2	5	5	8	2.40	
3124	2			0	2.49	
1031	3	4	4	8	4.12	
3081	3	3	3	3	3.97	
1044	4			3	2.80	
3121	4			7	4.06	
1077	5			3	3.70	
3122	5			6	5.16	
1099	6			8	3.36	
2975	6	4	4	4	5.47	
1175	7	7	7	16	7.39	
2394	7	2	4	4	7.12	+
1200	8	7	7	16	6.92	
2428	8	5	5	5	4.97	+
3044	8			2	2.49	
1291	9	4	4	4	4.83	
2396	9	5	5	13	5.98	
2398	9	5	5	8	4.08	
2474	10			9	5.53	
3001	10			7	4.64	
1893	10			2	2.14	
1312	11			7	4.62	
2611	11	4	4	9	4.64	
2970	11	7	7	9	3.45	
1348	12			0	2.46	
2973	12	2	2	4	5.29	
2974	12	5	5	11	5.83	
3002	13			3	4.03	
3003	13			4	3.19	
1405	13	5	5	10	2.43	
958	14			0	2.80	
2977	14	3	4	6	3.02	
3043	14			0	6.00	+
987	15	2	2	5	2.24	
2393	15	4	4	7	3.00	
2978	15			2	4.05	+
2402	16	4	4	4	7.41	
2401	16	3	3	3	2.10	
2473	16			0	4.05	
1591	17			6	3.19	
3171	17			0	4.10	+
2111	18	5	6	6	3.66	
2535	18			0	1.87	+
2138	19			1	2.16	
2461	19	2	2	7	4.68	
2162	20			2	4.28	
2185	21			0	2.43	
2395	21			3	1.17	
2219	22			3	2.14	
1996	23			0	1.73	
2021	24			2	2.87	+



FIGS. 1-5

considerable size (Fig. 2); they were present in all ovaries from the age of 2 months on, and had increased in number. Their presence gave the ovaries of the older animals a yellow color that was noticeable on gross examination.

Newly formed corpora lutea.—In those mice in which ova were present in the oviducts the new set of corpora lutea could always be differentiated from the older sets. In almost all instances their number corresponded with the number of ova present in the oviducts. In a few cases there were more newly formed corpora lutea than ova, but in all these the ova were near the distal end of the oviduct and some might already have entered the uterus.

Hyalinization.—Some characteristic changes occurred in corpora lutea of the dba mice that were never observed in C57 black mice. These alterations were first observed in the ovaries of 8 month old animals. In the central part of some of the old corpora lutea groups of cells became hyalinized (Fig. 3), as did also the peripheral areas later, and finally by coalescence the whole corpus became hyalinized (Fig. 4). These hyalinized bodies remained fairly large until the animals were about 19 months old, after which time gradual shrinkage, necrosis and, in many cases, extensive calcification occurred. The ovaries of all dba mice from 8 months on consistently contained such hyalinized corpora in varying number, whereas none were seen in ovaries from C57 black mice.

Number of corpora lutea.—As mentioned previously, the corpora lutea were counted in each ovary, a procedure that led to the following interesting observation. In the ovaries of the C57 black mice never more than 2 or 3 sets of corpora lutea were recognizable, whereas in the dba mice 7 or more sets persisted, so that the number of corpora lutea present was much greater in the dba animals (Figs. 4, 5).

The greatest number of corpora lutea counted in a C57 black mouse was 16 (mouse 1200, age 8 months). In the oviduct of this mouse there were 7 ova, and a corresponding number of newly formed corpora lutea could be identified in the ovary; the remaining 9 corpora very probably belonged to 2 previous sets of ovulations. In the dba animals the greatest number of corpora lutea that it was possible to identify was 35, but this was not the highest number reached; in some ovaries the whole organ appeared completely luteinized, and it was impossible to count the individual bodies.

The difference in the number of corpora lutea could have been caused by a difference in the number of follicles rupturing at a single ovulation. Although a slight difference did occur in this respect, it was not enough to account for the great dissimilarities that existed. The following evidence is offered to prove the statement above. As has been mentioned previously, all the ova present in the oviducts were counted. In the dba mice, ova were found in 18 oviducts, and a total of 93 was counted. The average number of ova liberated at a single ovulation was 5.2 (range, from 3 to 8). The oviducts of 23 C57 black mice contained a total of 97 ova, giving an average of 4.2 per ovulation (range, from 2 to 7). The oldest animals with oviducts containing ova were 19 months old in each strain.

Ovarian cysts.—Cysts were observed in the ovaries of 16 dba mice (30.7 per cent) and in 7 ovaries from mice of the C57 black strain (13.4 per cent), either multiple or simple and of varying size. The number of corpora lutea in cystic ovaries of the dba strain was not so great as in the noncystic ovaries. In one ovarian cyst of a C57 black mouse a papilliferous ingrowth of the epithelium was present.

Tumors.—Adenocarcinoma of the mammary glands was found in 3 dba females. One dba female had a fibrosarcoma of the vagina, and one C57 black female had a hepatoma.

Comparison of the size of the ovaries.—There were no appreciable differences in the approximate volumes of the ovaries of the two strains up to the age of 4 months, but after this the ovaries of the dba mice were much larger than those of the C57 blacks. This difference was due to the fact that the ovaries of the dba mice contained more corpora lutea. The approximate volume of each ovary is given in Tables I and II; the average values by months are shown in Fig. 6. The total average of all the ovaries of the dba strain was 8.47 cu. mm. and of the C57 black strain 3.62 cu. mm., which shows that the average size of the ovaries of dba mice examined was 2.3 times larger than that of the C57 black mice.

DESCRIPTION OF FIGURES 1 TO 5

Fig. 1.—Atretic follicle showing ovum surrounded by yellow lipochrome cells. C57 black ♀3124. Age 2 months. Mag. $\times$150.

Fig. 2.—Nodules composed of yellow lipochrome cells. High magnification of central area of Fig. 5. C57 black ♀3003. Age 13 months. Mag. $\times$ 150.

Fig. 3.—Hyalinized central cells of a corpus luteum. dba ♀2971. Age 8 months. Mag. $\times$ 150.

Fig. 4.—Ovary of dba ♀991 (age 15 months) showing many hyalinized corpora, some containing calcium granules. Compare in size with ovary of C57 black ♀3003, shown in Fig. 5. Mag. $\times$ 20.

Fig. 5.—Ovary of C57 black ♀3003. Age 13 months. Mag. $\times$ 20.

FIG. 6.—Average approximate volume of ovaries of dba mice (solid line) and C57 black mice (dotted line).

DISCUSSION

Taylor and Waltman (8), who studied the ovaries of dba and C57 black mice, found the average weight for the former strain to be greater than that for the latter. They examined 10 to 20 sections of each ovary and counted the number of corpora lutea in the largest available section, which was consistently greater in the ovaries of the dba mice, but their observation still left open the question as to the reason for the increased number of corpora lutea in the dba strain.

The present investigation showed two reasons for this: One was that on the average slightly more ovarian follicles ruptured at a single ovulation (5.2 in the dba, 4.2 in C57 black). The second, and main, reason was that sets of corpora lutea persisted longer in dba mice. In the C57 blacks the corpora lutea present at any one time represented not more than 3 sets; in dilute brown mice 7 or more sets may be present. The hyaline changes that occurred in the corpora lutea of the dba mice, but were never present in ovaries of the C57 black mice, were probably a result of the fact that these bodies persisted longer, regressed at a different rate, and showed a different type of degeneration.

According to Long and Evans (7) during pregnancy in the rat a profound retrogression and resorption of all pre-existing corpora lutea takes place, and on the 20th day of gestation only a single conspicuous set of corpora lutea is found, which are the corpora lutea of gestation.

Very little is known about the factors regulating the retrogression of the corpora lutea of estrus; evidently they are hereditary, as definite strain differences exist.

The growing follicles are the source of estrogen, and it can be considered that this hormone, whose effect on the development of the mammary gland is well known, is produced in slightly greater quantity in the ovaries of mice of the dba strain. Progesterone, product of the corpus luteum, is presumably elaborated in considerably greater amount; experimentally, it has only slight stimulating effect on the mammary gland (4). The combination of these two hormones in the proportions in which they are produced in the ovaries of dba mice, however, may very well influence the glands. This could produce dissimilarities in the mammary glands of the two strains that first become noticeable about the end of the third month, when there is a considerable difference in the number of corpora lutea and in the size of the ovaries (Tables I and II). In degree of development the mammary glands of young females of the two strains are similar. Taylor and Waltman (8) noted that differences first occurred at the end of the third month, when the main ducts

in dba mice were slightly more slender and the terminal branches definitely more numerous; later, more terminal branches and alveoli were constantly present. Van Gulick and Korteweg (5) also noted differences in the degree of development of the mammary gland in these two strains of mice, and compared its structure in C57 black mice to a tree in winter, and in the dba mice to a budding tree in the spring.

These inherent differences in the morphology and hormone production of different strains of mice should be taken into consideration in experimental studies concerning the effects of hormone injections.

SUMMARY

A comparative study of the right ovaries of virgin dba and C57 black mice revealed the following differences:

(a) The average number of ova escaping from one ovary at a given ovulation is 5.2 in dba and 4.2 in C57 black mice. As the growing follicles are the source of estrogen, it can be presumed that this hormone is produced in larger quantity in the dba mice.

(b) In C57 black virgin females 3 sets of corpora lutea are the most that can be found in an ovary. In the ovaries of dba mice the corpora lutea persist for a longer period, and 7 or more sets may be present. Consequently it can be presumed that a larger quantity of progesterone is produced in the ovaries of the dba mice.

(c) In older animals hyalin changes occur in the lutein cells of persistent corpora in dba mice, and in some ovaries large hyalinized areas are present; this alteration does not occur in the ovaries of C57 black mice.

(d) Groups of yellow lipochrome cells originating in corpora lutea atretica undergo atrophy and become dispersed in the ovarian stroma of dba mice, while in C57 blacks these cells hypertrophy and form aggregated nodules.

(e) Ovarian cysts occur more frequently in dba mice (30.7 per cent) than in C57 black mice (13.4 per cent).

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Factors Influencing the Stability of a Filtrable Agent of Chicken Leukosis and Sarcoma*

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Attempts to purify a filtrable agent of leukosis and sarcoma have met with little success, mainly because of its lability in partially purified preparations (14). The purpose of the experiments described in this paper was to study factors that would enhance the stability of this agent.

The agent used in this work (agent 13 of Stubbs and Furth) is able to produce leukosis, endothelioma, and sarcoma in chickens (22). Kabat and Furth (14) succeeded in concentrating it to only a moderate degree because of its lability after partial purification, and because of the presence of normal tissue components sedimentable at about the same speed as the agent. The soluble portion of the sediments obtained by centrifugating tumor extracts at 27,000 r. p. m. for 1 hour contained a variable fraction of the original activity and 0.6 to 8 per cent of the nitrogen of the original material (14, Table I). A more concentrated preparation was obtained when the extract was treated with sodium sulfate and centrifuged twice at high speed, but there was considerable loss of total activity as large quantities of the high speed sediments remained insoluble (14, Table VI).

Kirschbaum, Stern, and Hooker (15) centrifuged leukotic plasma or tissue extract one or two times at 30,000 r. p. m. The resuspended sediments carried part of, or all, the activity of the original material. Large amounts of these fractions were needed to produce leukosis, however, since the materials were injected undiluted.

Claude succeeded in isolating from Rous sarcoma I a highly purified fraction, the total activity of which was higher than that of the original tumor extract (9) because of the removal of inhibitory substances (7, 16). The minimum active dose was estimated to correspond to about 2,000 elementary granules of the agent. Particles of similar size were found in normal adult and embryonic chicken tissues (8).

Beard, Bryan, and Wyckoff (3) obtained by repeated centrifugation a purified virus-protein carrying most of the infective activity of rabbit papilloma. The absence of normal tissue component of similar size in their crude extract rendered their task easier. However, the 50 per cent infectious dose of the purified protein contained an estimated number of 94 million virus molecules.

The experiments to be described show that protein solutions have a nonspecific protective action on semi-purified preparations of agent 13.

MATERIALS AND METHODS

Animal inoculation.—Extracts or purified fractions of sarcoma 13 (22) were injected intramuscularly into barred rock chickens 5 to 25 days old. Two-tenths ml. were inoculated into each wing, leg, and breast, the injection site of each material being alternated in order to minimize the influence of local factors. Chickens were examined twice weekly, and the date of appearance of a definite nodule and the growth of the tumors recorded. Except for some with sarcomas at all injected sites that were killed for their tumors, the birds were kept under observation until death. The surviving chickens were killed 58 to 62 days after injection, since a longer survival would not have yielded a significantly larger number of tumors (12). All chickens were examined post mortem, and in doubtful cases the diagnosis was verified microscopically.

Preparation and high speed centrifugation of tumor extracts.—Extracts of sarcoma 13 were made from tumors developing in young birds either from cell grafts or after the injection of cell-free material. Tumors in birds that had received neutralizing sera were of low activity and so were not extracted. Since chicken sera may be inhibitory for agent 13 (12) the birds were usually bled to death in order that the tumors might contain only a minimal amount of blood. The sarcomas were excised under aseptic conditions, freed of necrotic parts, and extracted at once (extract No. 223) or after having been kept in sealed tubes in a chest containing solid carbon dioxide (extracts Nos. 607, 745, and 336).

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A weighed amount of pooled tumor was ground with sterile sand and extracted with an equal volume of a 0.005 *M* solution of phosphate buffer at pH 7.1. The suspension was cleared at about 3,000 r. p. m. in an angle centrifuge and the sediment extracted twice more with equal volumes of buffer, or only once with a double amount of buffer. The second extract yielded a larger amount of material sedimentable at high speed than the first (14). The supernatants from the successive extractions were combined, and incubated for 45 minutes at 37° C. with a hyaluronidase preparation to reduce the viscosity (14). This extract was then spun in an angle centrifuge at 3,000 to 4,400 r. p. m. until the turbidity disappeared. All operations were conducted under sterile conditions and, except for the enzyme incubation, at icebox temperature.

An air-driven quantity ultracentrifuge running in a vacuum was used (2). The head was chilled between the runs; the temperature during operation varied between 10° and 19° C. The lusteroid tubes were filled, as a rule, with 6.5 ml. fluid; its surface during centrifugation was at a distance of 5 cm. from the axis of rotation and the outermost portion of the bottom of the tube at a distance of 9.5 cm. All extracts submitted to high speed centrifugation were first spun at 8,000 r. p. m. for 15 minutes (3,580 to 6,800 times gravity). The supernatant of the 8,000 r. p. m. run, whose volume corresponded to about 2.3 times the weight of the tumor extracted, was termed "original extract."

The original extract was centrifuged at different speeds up to 27,000 r. p. m. (40,700 to 77,500 times gravity). Acceleration and deceleration times, varying from 10 minutes at 8,000 r. p. m. to half an hour at 27,000 r. p. m., were about equal. The supernatants were carefully pipetted off and the fatty top layer and the layer closest to the sediment, amounting together to 0.4 to 1 ml. per tube, were discarded. The tubes were inverted and allowed to drain, after which the sediments were broken up with a stirring rod and thoroughly titurated in a minimum amount of buffer, or buffer and serum, until a homogeneous suspension had been obtained. The same fluid was added to bring the suspension to the volume of the original extract, or to a known fraction of it. Operations involving high speed centrifugation were carried out without interruption and in the cold until injection of the final preparation.

Dilutions of preparations of agent 13 are expressed as a function of the volume of original extract from which they were obtained.

Nitrogen determinations were carried out by the micro-Kjeldahl method, with titration of the ammonia produced for amounts of nitrogen above 0.2 mgm. and with nesslerization for quantities of nitrogen from 0.007 to 0.05 mgm. Viscosity was measured with an Ostwald pipette.

Effect of solvent on agent 13.—An extract of sarcoma 13 (No. 223) prepared with saline was filtered through a Berkefeld "V" filter, and stored at −60° C. in small tubes; samples were thawed as needed. The filtered extract was diluted with serum or tumor extract to the lowest dilution indicated in the tables (Tables III and IV), and the mixtures were incubated 45 minutes at 37° C., kept 45 minutes at room temperature, and then overnight in the icebox. Serial dilutions in phosphate buffer were made immediately before injection.

The extracts of sarcomas 13 and 16 (12) used as solvents were prepared as previously described, but the enzyme treatment was omitted. A portion was inactivated by heating for 45 minutes in a water bath at 60° C. Both fresh and inactivated extracts were tested for infectivity.

Fresh rabbit and guinea pig sera were obtained immediately before an experiment. For inactivation the sera were heated for 30 minutes at 55° C. Titrations of complement in the sera, or in the mixtures of serum and sarcoma 13 extract, were made after incubation for 45 minutes at 37° C. and 45 minutes at room temperature, by adding 0.4 ml. of saline and 0.2 ml. of a 5 per cent suspension of sensitized sheep erythrocytes to 0.2 ml. of a serum dilution. The mixtures were incubated for 30 minutes at 37° C. and kept overnight in the icebox. The titer of complement is the highest dilution showing complete or almost complete hemolysis.

EXPERIMENTAL

Latent Period Values as a Measure of Activity

The activity of a preparation of agent 13 can be expressed by the smallest amount able to produce tumors. The value thus obtained is based on the few animals susceptible to the highest dilution. As a variation of individual or local susceptibility could easily result in a tenfold variation in the values obtained, it is desirable to supplement this value by a figure that measures smaller differences of activity, and is based on all tumors obtained. Such a method, based on values of latent periods, has been developed by Bryan and Beard for the estimation of activity of the rabbit papilloma virus-protein (5), and has been applied to agent 13.

The average latent period is defined as the average time between inoculation and the discovery of a tumor by palpation. It has been shown that this value may be used as a measure of the quantity of agent 13 injected (12). In general, there is a linear relationship between the logarithm of the amount of agent and the average latent period; actually, the curve is slightly

S-shaped, since the increase of average latent period is less in both extreme ranges. This has previously been discussed for small amounts of agent (12). On the other hand, very large amounts of agent seem unable to produce a tumor in less than about 6 days. The flattening of the curve for large doses is indicated by the values of Tables II and V. For practical purposes a straight line intersecting the S-shaped curve expresses satisfactorily the relation between dose and latent period. The slope of this line depends upon the solvent used. An average increase of latent period of 7.2 days for a tenfold dilution in saline (or buffer) is derived from data previously published (12, Table VIII, normal chicken sera), which are in good agreement with recent experiments. A smaller increase of mean latent period is found when an agent 13 preparation is diluted with buffer containing 10 per cent rabbit serum; an average increment of 3.2 days is obtained from the combined values of Table V.

An illustration of the influence of the solvent and of the smaller increase of latent period in the lower range is given also by the results of injection into chicks of an extract from sarcoma 13 grown in ducklings (12). The mean latent periods at dilutions 1:2, 1:20, 1:200, and 1:2000 in buffer were respectively 9, 10, 14.8, and 28 days; no tumors were obtained with a 1:20,000 dilution in buffer. Dilutions 1:2 to 1:20,000 in buffer with serum produced tumors after 7, 8.5, 10.6, 14.9, and 16.6 days, respectively.

If a straight line relationship between latent period and logarithm of dosage is assumed, each preparation of agent 13 can be characterized by two values. One is the average latent period of all tumors produced at all dilutions; the other, the corresponding average quantity of active agent. The logarithm of this is given by the average of the logarithms of the amounts of preparation that produced a tumor. These two values permit the comparison of activity of two preparations diluted in the same solvent, provided either the average latent periods or the average active amounts are the same. As this is usually not the case, it is convenient to calculate the amounts of material of equivalent activity that would produce tumors in a given average latent period. This value of average latent period used as a reference is chosen to correspond to an amount of agent 13 giving 50 per cent positive inoculations; this gives a clearer picture of the unit of activity defined by latent periods.

The latent period of the 50 per cent response dose was determined for each experiment by plotting the percentage of successful inoculations expressed in probits [1] against the logarithm of the dilution injected. The dilution corresponding to a probit value of 5 was determined graphically. The corresponding latent period was derived from the relation between dilution and latent period. Twenty-two days is a satisfactory average value for the experiments reported in this paper; a higher value was given by the tests with extract No. 679 previously described (12).

The logarithm of the dilution causing tumors in 22 days and likely to give a 50 per cent positive response is given by the formula:

$$-\log.\,\mathrm{dil}_{50} = -\log.\,\mathrm{dil}_{\mathrm{av.}} + \frac{22 - \mathrm{lat.\,per.\,av.}}{k}$$

Cf. formula 4 of Bryan and Beard (5).

The value of k is 7.2 for saline or buffer solutions and 3.2 for dilutions in serum-buffer mixtures. Values of 50 per cent response doses are given in Table V, together with the experimental data from which they were calculated.

The 50 per cent dose defined by the latent period is not directly related to the percentage of positive inoculations at each dilution of the experiment. The 50 per cent values, calculated directly from the number of tumors according to the method of Reed and Muench (18) or determined from a plot of probit against dilution, were different from those derived from the latent periods. They allowed, however, the same conclusions.

Speed Required to Sediment Agent 13

As the centrifugal field to which Kabat and Furth (14) submitted agent 13 (41,000 to 77,000 times gravity) was considerably higher than that employed by Claude and Rothen (9) for the Rous agent (17,500 times gravity), a preliminary experiment was performed to determine the optimal speed for concentration of agent 13.

The 8,000 r. p. m. supernatant of an extract from sarcoma 13 (No. 607) was spun for 1 hour at speeds varying from 12,000 to 27,000 r. p. m. The sediments, supernatants, and also the sediment of the 8,000 r. p. m. centrifugation were taken up in buffer to the volume of the corresponding original extract. Three successive dilutions in buffer of the products (1:1 to 1:100, or 1:10 to 1:1,000) were each injected at 9 sites.

Table I shows that according to 50 per cent response values only about one-tenth of the original activity was left in the supernatant of the 12,000 r. p. m. centrifugation, about one-hundredth in the 15,000 r. p. m. supernatant, and one-thousandth in the 27,000 r. p. m. supernatant. The values of the minimal infective dose also indicate a strong decrease of activity of the supernatant. The smallest dose of original extract tested (dilu-

[1] Probit units were introduced by Bliss (4) for transforming the usual sigmoid dosage-mortality curve to a straight line. The probit value of a 50 per cent incidence is 5. A table of probits corresponding to any percentage value and theory of the use of this unit will be found in the paper of Bliss (4).

tion 1:1,000) produced 5 tumors at 9 sites after an average of 18.2 days, whereas the smallest dose of the 12,000 r. p. m. supernatant (same dilution) gave rise to only 1 tumor after 28 days at 9 sites inoculated. Very little seemed thus to be gained by spinning a tumor extract above 15,000 r. p. m. in order to clear it of agent 13. The centrifugal field developed at that speed (12,600 to 23,900 times gravity) was similar to that used in work with the Rous sarcoma I agent (9).

obtained at the highest speeds showed the largest total infectivity.

Action of Tumor Extracts and of Sera on Agent 13

To test whether a deleterious action of the solvent could explain the previous results, the experiment summarized on Table II was set up. The 8,000 r. p. m. supernatant of an extract, No. 745, was spun for 1

Table I: Activity of Fractions of Sarcoma 13 Obtained by Centrifugation at Different Speeds

Fraction of sarcoma 13 extract	Nitrogen per ml. (resuspended in buffer to original volume), mgm.	Nitrogen of smallest active dose, mgm.	Nitrogen of 50% dose (latent period = 22 days), mgm.
Supernatant			
" 8,000 r.p.m. (original extract)	2.12	4.2×10^{-4}*	9.3×10^{-4}
" 12,000 r.p.m.	2.11	4.2×10^{-4}*	9.8×10^{-3}
" 15,000 r.p.m.	1.55	3.1×10^{-3}*	1.1×10^{-1}
" 20,000 r.p.m.	1.67	3.3×10^{-2}	1.2×10^{-1}
" 27,000 r.p.m.	1.89	3.8×10^{-1}	8.4×10^{-1}
Sediment			
" 8,000 r.p.m. (from 3,000 r.p.m. supernatant)	0.034	6.8×10^{-4}	1.1×10^{-2}
" 12,000 r.p.m.	0.079	1.6×10^{-4}*	4.5×10^{-3}
" 15,000 r.p.m.	0.162	3.2×10^{-4}*	7.3×10^{-3}
" 20,000 r.p.m.	0.188	3.8×10^{-4}	3.9×10^{-3}
" 27,000 r.p.m.	0.286	5.7×10^{-4}	3.9×10^{-3}

In this experiment (Exper. 1) the chickens were 10 days old at the time of injection. All fractions were prepared from extract No. 607.
* A higher dilution was not tested.

Table II: Protective Action of Serum and Inactivated Supernatant on Agent 13

		Fraction to original volume and diluted in					
		Buffer		Buffer + 10% rabbit serum		Inactivated supernatant	
Fraction injected	Dilution	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Ratio: No. tumors / No. sites inoculated	Average latent period, days
8,000 r.p.m. supernatant	1:10	6/10	15.7	6/10	17.5	7/10	17*
(= original extract)	1:100	3/10	21	6/10	19.2	4/10	18.4
(1.64 mgm. N/ml.)	1:1000	5/9	30.8	5/9	25.9	3/9	29.2
	1:10000	0/9		4/9	29.7	2/9	24.5
15,000 r.p.m. sediment	1:1	7/9	13.5	6/9	11.1	7/9	11†
(0.028 mgm. N/ml.)	1:10	7/9	16	8/9	14.4	7/9	13.5
	1:100	0/9		6/9	14.6	5/9	16.8
	1:1000	0/9		3/9	24.5	4/9	24.5
15,000 r.p.m. supernatant	1:1	13/18	14.8	See under Buffer			
(1.63 mgm. N/ml.)	1:10	7/9	14.5	7/9	15		
	1:100	5/9	21.7	5/9	21.7		

In this experiment (Exper. 2) the chickens were 22 days old at the time of injection. All fractions were prepared from extract No. 745.
* Eighteen sites injected with 0.2 ml. undiluted inactivated 8,000 r.p.m. supernatant alone showed no sarcoma.
† Eighteen sites injected with 0.2 ml. undiluted inactivated 15,000 r.p.m. supernatant alone showed no tumor.

The total activity of each sediment was, however, only a small fraction of the amount of agent removed from the corresponding supernatant. The activity per mgm. nitrogen of the several sediments was not higher than that of the original extract. Agent 13 thus appeared to be inactivated in the sediment either because of the use of an excessive centrifugal field or because of a deleterious action of the solvent. The first hypothesis was not very likely, as the loss of total activity of combined sediment and supernatant occurred at all speeds. Furthermore, the sediments

hour at 15,000 r. p. m. The sediment was washed and resuspended at the original volume either with buffer, or with buffer containing 10 per cent inactivated normal rabbit serum, or with the corresponding supernatant that had been inactivated. Serial dilutions were made in the same fluids. The 8,000 r. p. m. and 15,000 r. p. m. supernatants were also diluted in the different fluids.

Should the results of injection of the buffer dilutions only be considered, the activity of the 15,000 r. p. m. sediment estimated by end titers would appear to be

one-hundredth that of the original extract (Table II). However, the original extract and the 15,000 r. p. m. sediment resuspended in diluted rabbit serum had about equal activity, as indicated by the number of tumors produced; the latent period values even showed an increased activity of the sediment. The 15,000 r. p. m. supernatant seemed about as active as the original extract, but end titers were not reached.

The difference of infectivity of solutions in buffer or in buffer—serum was more definite for the sediment than for the original extract or for the supernatant. The difference was also more pronounced at high than at low dilutions. This suggested that the inactivation of agent 13 suspended in buffer occurred when the protein level became low. Results obtained with inactivated supernatant as a solvent, indeed, duplicated those given by suspensions of agent in diluted serum.

periods at dilution 1:20 were 11.5 and 15 days respectively. Heating for 45 minutes at 60° C. did not destroy completely the activity of one of these extracts, although this treatment had been sufficient in Experiment 2. A sarcoma developed after 22 days at 1 of the 3 sites injected with the undiluted heated extract No. 935.

Despite this trace of activity, a standard preparation of agent 13 was not more active after incubation with the heated extract from sarcoma 13, than after contact with the inactivated extract from sarcoma 16 (Experiment 3a). The same degree of protection was afforded by two tumor extracts and by a solution of inactivated rabbit serum (Experiment 3b). A fresh extract from sarcoma 16 (Experiment 3a), however, was slightly inhibitory. The number of tumors elicited was the lowest at each dilution, and the mean latent period

TABLE III: EFFECTS OF TUMOR EXTRACTS ON AGENT 13

Agent 13 incubated with:	Ratio: No. tumors / No. sites inoculated	Average latent period ± standard deviation, days	Ratio: No. tumors / No. sites inoculated	Average latent period ± standard deviation, days
Dilution of sarcoma 13 extract	1:20		1:200	
Sarcoma 16 extract No. 517, not inactivated	9/12	15.6 ± 4.6	8/12	17.1 ± 4.7
" 16 " " 517, inactivated	10/12	11.9 ± 1.8	11/12	19.4 ± 9.7
" 13 " " 935, "	12/12	12 ± 2.3	11/12	17.5 ± 11.4
Dilution of sarcoma 13 extract	1:100		1:1000	
Sarcoma 16 extract No. 386, inactivated	6/10	19.2 ± 4.8	9/12	23.8 ± 12.3
" 13 " " 481, "	8/10	17.1 ± 5.7	8/12	18.6 ± 6.7
Buffer + 10% inactivated rabbit serum	8/10	18.2 ± 5.1	7/12	24.8 ± 8.9

In the first experiment (Exper. 3a) the chickens were 25 days old at the time of injection; in the second experiment (Exper. 3b), 19 days old. All were injected with sarcoma 13 extract No. 223.

The protective action of the inactivated supernatant thus appeared nonspecific.

A chemically induced chicken tumor (sarcoma 16) containing an antigen related to that of agent 13 has been previously described (12). As this tumor cannot be transmitted by filtrate the question arose whether it would contain an inhibitory factor for agent 13 or lack the protective property of extracts from sarcoma 13 (Table II). The inactivation of a virus by extracts of sarcoma 16 might explain the failure to demonstrate an agent causing this growth.

The effect of varied tumors extracts on agent 13 is shown in Table III. Two extracts from sarcoma 16 (Nos. 517 and 386) were prepared from tumors that had given metastases, in order to avoid a neutralizing activity of the serum contained in the tumor. Previous experiments have shown that the sera of birds in which sarcoma 16 had metastasized do not possess neutralizing antibodies against agent 13 (12). As expected, extracts from sarcoma 16 produced no tumor when injected into newly hatched or 2 day old chicks. Before heat inactivation, the extracts from sarcoma 13 (Nos. 935 and 481) were highly active; the average latent of the tumors produced by dilution 1:20 differed significantly from the corresponding values for the inactivated extracts ($D = 3.7 \pm 1.65$ and 3.6 ± 1.68, respectively [2]). The slight inhibition effected by the fresh extract of sarcoma 16 seems insufficient, however, to account for the neutralization of a hypothetical virus that it might contain.

It seems more likely that the slight neutralizing action of the fresh extract of sarcoma 16 is analogous to that of fresh normal sera (Table IV). This action of complement or of a heat-labile factor of serum is of special interest, in view of previous reports on neutralization of extracts of Rous sarcoma I by antisera to normal fowl tissue only in the presence of complement (1, 13).

A preliminary experiment showed that agent 13 produced more tumors, and was active at higher dilutions, when it was incubated with inactivated normal or nonspecific sera containing inactivated guinea pig

[2] The standard error of a difference and chi square values were calculated according to formulas for small numbers. The statistical methods have been described (12).

serum, than when incubated with these sera containing fresh guinea pig serum.

The action of fresh and inactivated normal sera alone was also tested (Table IV). The rabbit serum containing the largest amount of complement of 3 normal sera was chosen for this experiment. Its complement titer was low (1:2) as compared to fresh guinea pig sera (1:32 in Experiment 4a and 1:64 in Experiment 4b). After incubation with agent 13 the complement titers in the mixtures were 1:1 for rabbit serum, and 1:32 and 1:64 for guinea pig serum. The diminution of activity of agent 13 after incubation with the fresh sera of both species was about equal.

"milk factor." Rabbit 118 received intravenous injections of the soluble portion of the sediment. Rabbit 119 was injected with "washings," representing a layer of loosely packed material above the sediment. Complement fixation tests gave high titers with both sera (1:800 and 1:1,600). The same amounts of protein were used for immunization with this material as for the preparation of rabbit antisera to sarcoma 16 (12).

The previous experiments suggested a slight deleterious action of a heat-labile fraction of serum for agent 13, but did not demonstrate it conclusively. Thus it seemed advisable to inactivate sera used to resuspend labile preparations of agent 13.

Table IV: Effect of Fresh and Inactivated Sera on Agent 13

Agent 13 incubated with:	Ratio: No. tumors / No. sites inoculated	Average latent period ± standard deviation, days	Ratio: No. tumors / No. sites inoculated	Average latent period ± standard deviation, days	Ratio: No. tumors / No. sites inoculated	Average latent period ± standard deviation, days
Dilution of sarcoma 13 extract...	1:20		1:200		1:2000	
Buffer	8/12	19.9 ± 6.2	2/12	21	0/12	
Fresh normal rabbit serum No. 1	6/9	20.4 ± 8.7	1/9	21	2/9	17.5 ± 0
Fresh normal G. P. serum	7/12	19.5 ± 7	1/12	14	1/12	24.5
Inactivated normal rabbit serum No. 1	9/9	15.9 ± 3.5	2/9	15.7 ± 2.5	2/9	17.5 ± 4.9
Inactivated normal G. P. serum	10/12	14.8 ± 2.9	2/12	21 ± 4.9	3/12	23.3 ± 8.1
Dilution of sarcoma 13 extract...	1:50		1:500		1:5000	
Fresh normal G. P. serum	4/9	17.5 ± 6.4	3/10	17.5 ± 6.1	2/9	35 ± 4.9
Inactivated normal G. P. serum	4/9	16.6 ± 9.4	7/10	19.5 ± 7.5	4/9	24.5 ± 10.3
Inactivated normal rabbit serum No. 118	6/9	15.2 ± 5.3	8/10	18.8 ± 8.1	5/9	20.3 ± 8.4
Inactivated rabbit serum No. 118 anti-mouse mammary tumor	5/9	16.8 ± 4.6	7/10	17.5 ± 4.9	3/9	25.7 ± 10.1
Inactivated rabbit serum No. 119 anti-mouse mammary tumor	5/9	16.8 ± 1.6	7/10	20.5 ± 6.8	3/9	26.8 ± 16.2
Inactivated rabbit serum No. 95 anti-sarcoma 16	0/9		0/10		0/9	

In the first experiment (Exper. 4a) the chickens were 5 days old at time of injection; in the second (Exper. 4b), 18 days old. All were injected with sarcoma 13 extract No. 223.

In Experiment 4a the combined latent period values for fresh and inactivated sera showed a significant difference at dilution 1:20 ($D = 4.6 \pm 2.21$); the variation in the number of tumors was below the limit of significance $\chi^2 = 3.3$). Suspensions of agent 13 in buffer were about as active as those in fresh sera, but the end dilution was reached earlier.

In Experiment 4b a difference between fresh and inactivated guinea pig sera was very doubtful. Both fresh and inactivated guinea pig sera appeared to give somewhat less protection to agent 13 than inactivated rabbit sera. An inactivated rabbit antiserum to sarcoma 16 used as a control neutralized completely agent 13 (12), whereas 2 rabbit antisera to a mouse mammary tumor lacked neutralizing power.

The antisera to mammary tumor of mice were obtained as follows: An extract from a mammary tumor transmitted in C3H mice (11) was centrifuged for 2 hours at 27,000 r. p. m. This tumor originated in a C3H × Ak hybrid, and presumably contained a

Isolation of Active Fractions from Sarcoma 13

The results in Table II show that inactivated serum protected agent 13 as well as did supernatant fluid. Keeping the resuspended sediment in concentrated solution would probably also afford protection to the agent.

Accordingly, in the next experiment, the sediment of an extract from sarcoma 13 (No. 336) that had been centrifuged for 1 hour at 15,000 r. p. m. was resuspended in buffer to one-tenth the original volume, cleared of insoluble particles at 4,400 r. p. m. in an angle centrifuge, spun again at 15,000 r. p. m., and resuspended in buffer to one-twentieth of the original volume; once more centrifuged at low and high speeds, and brought to one-sixtieth of the original volume.

The second and third high speed runs were reduced to 45 minutes. Samples of the different suspensions of high speed sediments (before the low speed run) were kept in the icebox. All serial dilutions were made

at the same time in buffer containing 10 per cent inactivated rabbit serum. In this way a 200-times concentration of agent 13 was obtained, as indicated by the latent period values (Table V). The total activity of the several sediments did not differ within the limits of experimental error from that of the extract from which they were derived. If the total infectious activity of the original extract estimated by latent periods is represented by 100 that of the first sediment would be 130; that of the second, 22; and that of the third, 62.

TABLE V: ACTIVITY OF PURIFIED FRACTIONS OF SARCOMA 13

Fraction of sarcoma extract No. 336	8,000 r.p.m. supernatant (= original extract)		First 15,000 r.p.m. sediment		Second 15,000 r.p.m. sediment		Third 15,000 r.p.m. sediment	
	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Ratio: No. tumors / No. sites inoculated	Average latent period, days
Dilution (based on original volume)								
10:1	.....	....	.....	....	.....	....	12/12	8.7
1:1	5/6	11.2	6/6	12.2	12/12	13.1	12/12	10.8
1:10	5/6	14	10/12	12.9	9/12	12.1	8/12	15.3
1:100	12/12	13.7	8/12	13.6	9/10	17.9	6/12	18.7
1:1,000	10/12	19.6	7/10	22.5	8/12	24.9	4/12	17.5
1:10,000	3/10	29.2	1/12	14	1/6	35	3/12	19.8
1:100,000	1/12	42	0/6		0/6		.....	....
N per ml. of original volume, mgm.	1.44		0.0227		0.0055		0.0043	
N of smallest active dose, mgm.	2.9×10^{-6}*		4.5×10^{-7}		1.1×10^{-7}		8.6×10^{-8}*	
N of 50% dose (latent period = 22 days), mgm.	6.6×10^{-5}		8.0×10^{-7}		1.1×10^{-6}		3.2×10^{-7}	

In this experiment (Exper. 5) the chickens were 20 days old at the time of injection.
* A higher dilution was not tested.

DISCUSSION

Repeated high speed centrifugations of an extract from sarcoma 13 and maintenance of a sufficient level of protein during the process make it possible to obtain concentrated preparations of agent 13 carrying all or most of the infectivity of the original material. The smallest active dose contained 8.6×10^{-8} mgm. of nitrogen. The best preparation of agent 13 previously obtained (14) had an activity of 6×10^{-6} mgm. of nitrogen, whereas the smallest infective unit of Rous sarcoma I obtained by Claude (9) had a dry weight of 4×10^{-10} mgm. The degree of purification (335 times) was of the same magnitude as in Claude's experiment (425 times). The higher potency of the preparation of Rous sarcoma I is thus referable to the higher infectivity of the tumors extracted, and to an increase of activity following the removal of inhibitory substances. The small difference in protein content of the second and third sediments of Experiment 5 suggests that little would be gained by additional centrifugation. Purification of the Rous agent and of the influenza virus by adsorption and by chemical methods proved less effective than differential centrifugation (6, 9, 19). The best chance of obtaining more concentrated preparations of agent 13 would probably be to work with a more active tumor extract, in which the proportion of agent to normal tissue components would be higher. However, the extract used in Experiment 5 was among the best obtained. The only extract of much higher potency came from grafts of sarcoma 13 in newly hatched ducklings (12), but the close of the hatching season for ducklings prevented us from using such material for differential centrifugation.

The facts that agent 13 was progressively removed from the supernatant at varied centrifugation speeds and that traces of it were still present in the 27,000 r. p. m. supernatant (Table I) are not indicative of differences in size of the agent particles. According to the theory of sedimentation in an angle centrifuge, developed by Pickels, convection currents are responsible for a residual activity of the supernatant and for a greater sedimentation rate (17). The application of Pickels' formula to the present data concerning viscosity of extract and centrifugation time gives values of 100 or 110 mμ, but the particle diameter is smaller than these figures, as Pickels' formula applies to a medium where a synthetic density gradient counteracts convective disturbances. The calculated values are thus in agreement with those indicated by optical methods (20, 21) for the Rous agent (70 mμ) and the leukosis agent 1 (72 mμ). Assuming a diameter and nitrogen content for agent 13 similar to those of the Rous agent (8), the smallest infective dose of agent 13 obtained would contain about 4.3 million elementary particles.

It was shown that purified preparations of agent 13 lose their infectivity in solutions devoid of protein. Claude resuspended a purified preparation of Rous agent in a volume of buffer corresponding to 1.5 times the weight of tumor extracted, and made serial dilutions in a buffer containing 2 per cent rabbit serum (9), for it is a routine procedure in many laboratories to make suspensions of viruses in serum or broth. The nature of the inactivation in the absence of protein

is not clear. Aggregation of macromolecular material probably occurs spontaneously and may be favored by the absence of protective colloid, as is suggested by the variable proportion of sedimentable material obtained by high speed centrifugations of extracts of sarcoma 13. The agent of leukosis 1 loses its filtrability after high speed centrifugation (15). Aggregation also may be responsible in part for the loss of activity of suspensions of agent 13 in buffer solutions.

SUMMARY

A filtrable agent of leukosis and sarcoma of chickens (agent 13) was sedimented from tumor extracts at a speed of 15,000 r. p. m. in an angle centrifuge. When resuspended and diluted in buffer the sediments contained only a small fraction of the infectivity of the original extracts. The total activity was recovered when the sediments were kept in concentrated solution, or resuspended and diluted in buffer containing inactivated rabbit serum. A preparation of agent 13 purified by 3 successive centrifugations at 15,000 r. p. m. was infectious in amounts containing 8.6×10^{-8} mgm. of nitrogen. The 50 per cent response dose (calculated from latent period values) contained 3.2×10^{-7} mgm. of nitrogen.

Inactivated supernatants of extracts from sarcoma 13 did not afford a better protection than solutions of inactivated serum. Inactivated extracts of a nonfiltrable chicken tumor (sarcoma 16) had no inhibitory action on agent 13. Thus the protective action of protein solutions appeared to be nonspecific.

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Abstracts

Reports of Research

An Induced Carcinoma in the Fowl. BIELSCHOWSKY, F., and GREEN, H. N. [Univ. of Sheffield, Sheffield, England] *Nature, London,* **156**:780. 1945.

Five Rhode Island Red cocks received 2-acetylaminofluorene in the food (5.5 gm. in 45 weeks). Three died during the first year without tumors. The remaining two were killed in the 87th week. One showed an atypical leukemia, the liver being almost wholly replaced by immature blood cells. In the other, both kidneys were largely replaced by nodules of invasive anaplastic carcinoma.—E. L. K.

A Metabolite of 2-Acetamidofluorene. BIELSCHOWSKY, F. [Univ. of Sheffield, Sheffield, England] *Biochem. J.,* **39**:287-289. 1945.

When rats received 4 to 6 mgm. of 2-acetamidofluorene in the food, they excreted 5 to 8% of this amount in the urine as the 7-hydroxy derivative; this compound, which gives a strong color reaction with nitrite, was identified by comparison with a synthetic specimen. The excretion of the hydroxy derivative ceased 2 to 3 days after withdrawal of the parent compound from the diet, independently of the duration of administration.—E. L. K.

The Preparation of 2-Amino-7-hydroxyfluorene. GOULDEN, F., and KON, G. A. R. [Chester Beatty Research Inst., Roy. Cancer Hosp. (Free), London, England] *J. Chem. Soc.,* 930. 1945.

A method is described for the synthesis of 7-hydroxy-2-acetamidofluorene, which is a metabolic product of the carcinogenic 2-acetamidofluorene (see preceding abstract). —E. L. K.

Factors Affecting Carcinogenesis. III. The Effect of Hydrogenation of Lipid Solvents on Carcinogenesis by 3,4-Benzpyrene. DICKENS, F., and WEIL-MALHERBE, H. [Brit. Emp. Cancer Campaign, Roy. Victoria Infirmary, Newcastle-upon-Tyne, England] *Cancer Research,* **6**:161-170. 1946.

Four groups of mice were given subcutaneous injections of a single dose of 3,4-benzpyrene dissolved in 50% solutions in tricaprylin of the following: cod liver oil, hydrogenated cod liver oil, mouse fat, hydrogenated mouse fat. The incidence of tumors with cod liver oil was about the same as that found for tricaprylin, which has been adopted as standard solvent. There was a significantly higher incidence in the mouse fat series, whether hydrogenated or natural solvent was used, than in the two cod liver oil solvents. Hydrogenation had a tendency to decrease the tumor incidence in the cod liver oil series, but the difference was not significant. In the mouse fat series, hydrogenation caused a significantly increased incidence. The latent period tended to be shorter with unsaturated fats. The rate of elimination was determined for these solvents. It was accelerated in the unsaturated solvents, but after their hydrogenation the rate was slowed down to that observed with tricaprylin. The fact that the sample of mouse fat used in these experiments was not anticarcinogenic was confirmed by repeating the test with the same sample undiluted with tricaprylin. Tricaprylin behaved as an entirely neutral diluent. The lack of inhibitory action of the present sample of mouse fat is attributed mainly to its much lower content of phospholipins than the sample previously used by the authors.

From a consideration of these results it is concluded that: (a) The anticarcinogenic action observed by others as well as by the authors in certain samples of mouse fat is due neither to their content of highly unsaturated fatty acids (present as glycerides) nor to the presence of fully saturated ones. (b) The rate of elimination of benzpyrene in these experiments bore no direct relationship to the cocarcinogenic or anticarcinogenic nature of the solvent, but it is pointed out that this may be because of complication by physical factors. One consistently observed correlation was the shortening of the latent period accompanying rapid elimination.—Authors' summary.

Factors Affecting Carcinogenesis. IV. The Effect of Tricaprylin Solutions of Cholesterol and Phospholipins. WEIL-MALHERBE, H., and DICKENS, F. [Brit. Emp. Cancer Campaign, Roy. Victoria Infirmary, Newcastle-upon-Tyne, England] *Cancer Research,* **6**:171-178. 1946.

Three groups of mice were injected subcutaneously with a single dose of 0.3 mgm. of 3,4-benzpyrene dissolved in: (a) tricaprylin, (b) tricaprylin containing 1.5% lecithin and 1.5% cephalin, (c) a 3% solution of cholesterol in tricaprylin. Observations were made of tumor incidence and rate of elimination of benzpyrene. Tricaprylin was taken as the standard solvent for comparison with the others. The tumor incidence at 20 and 30 weeks was: cholesterol series, 79 and 82%; tricaprylin, 38 and 46%; phospholipins, 12 and 47%, respectively. The increased incidence with cholesterol is highly significant ($P<0.01$), but the retardation with phosphatides is not statistically proved ($P=0.1$) and a larger number of observations would be required to establish it. From a consideration of all the evidence it appears probable, nevertheless, that the inhibitory action of phospholipins on carcinogenesis is genuine. The anticarcinogenic effect observed by several authors for various samples of animal fats might well be due to their content of phospholipins. The complex composition of most natural oils makes it preferable to use pure synthetic vehicles where possible, as otherwise the interpretation of results is hardly possible (*e.g.,* with

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cod liver oil). It is shown that, compared with tricaprylin, the solvents (arachis and sesame oils) previously used by the authors and others for comparison with mouse fat as solvent, are themselves cocarcinogenic. The differences in carcinogenic activity previously attributed to the anti-carcinogenic activity of mouse fat are in part due to this fact.

The rate of elimination of benzpyrene is accelerated when the solvent contains cholesterol ($k=0.027$), and inhibited in the phospholipin solution ($k=0.0062$), compared with tricaprylin ($k=0.016$); the differences in these rates are highly significant ($P<0.01$). The fact that the more rapid elimination of benzpyrene is associated with higher carcinogenic activity, and slower elimination with lower activity, leads the authors to suggest that, contrary to frequently expressed opinion, the rapidity of elimination of the carcinogen is associated with high carcinogenic activity. The authors suggest that the oxidative metabolism of the carcinogenic hydrocarbon may be a necessary condition for its carcinogenic activity. If this is so, it is probable that an oxidative metabolite of the hydrocarbon is the true carcinogen rather than the hydrocarbon itself.—Authors' summary.

Effects of Implantation of Methylcholanthrene in the Brain of the Dog. Bailey, P., Shimizu, K., and Davis, E. W. [Coll. of Med., Univ. of Illinois, Chicago, Ill.] *J. Neuropath. & Exper. Neurol.*, **3**:184-188. 1944.

Methylcholanthrene implanted in the cerebrum of 6 basis of which 3 lesions of fibrosarcomatous appearance developed. No gliomas were induced.—A. Cnl.

X. Carcinoma of Mammary Gland Following Injection of Methylcholanthrene into Mice of NHO Strain. Strong, L. C. [Yale Univ., New Haven, Conn.] *Proc. Soc. Exper. Biol. & Med.*, **59**:217-220. 1945.

One hundred and twenty-five female mice belonging to 2 sublines of the NHO strain were injected subcutaneously at 60 days of age with 1 mgm. of methylcholanthrene. This strain is highly resistant to spontaneous tumors. Following treatment with the carcinogen 75 mice (60%) developed carcinoma of the mammary gland. These induced mammary tumors duplicated in histologic detail the tumors arising spontaneously or after injection of an estrogen (A or C3H mice). In addition, the methylcholanthrene-induced tumors exhibited 4 characteristics not evident, or less pronounced, in spontaneous tumors: (1) there was an increased tendency to undergo squamous metaplasia, (2) many tumors (18%) underwent considerable anaplastic change, (3) these anaplastic tumors invaded normal tissues extensively, (4) they metastasized to bone. These carcinogen-induced tumors appear to resemble, in some respects, human mammary carcinoma. Some of the factors involved in the origin of spontaneous mammary tumors are briefly discussed.—M. B.

Action of Methylcholanthrene on Certain Scars of the Skin in Mice. Lacassagne, A., and Latarjet, R. [Lab. Pasteur de l'Inst. du Radium, Paris, France] *Cancer Research*, **6**:183-188. 1946.

In newborn and adult mice methylcholanthrene was applied to cutaneous fields that had formerly undergone either ultraviolet irradiation with 1,500 finsens, or the removal of a cutaneous disk. Painting a cutaneous wound with methylcholanthrene affected neither the mode nor the duration of repair. A skin zone free from hair follicles and sebaceous glands appeared refractory to the carcinogenetic action of methylcholanthrene. On the other hand, a skin zone repaired after photodermatitis under conditions such that some hair follicles with sebaceous cells had been restored or newly formed, gave rise to rapidly evolving epitheliomas if treated with methylcholanthrene. These experiments emphasize the role of the hair follicles and sebaceous glands in the origin of cutaneous epitheliomas induced by chemical substances, and the high carcinogenic potential of the proliferating strip that underlies the process of repair.—Authors' summary.

Influence of Age on Total Epidermal Lipid During Carcinogenesis Induced by Methylcholanthrene in Mice. Suntzeff, V., Cowdry, E. V., and Carruthers, C. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research*, **6**:179-182. 1946.

Investigations were carried out to determine the role of the total lipid of mouse epidermis as a factor in explaining the difference in response of the epidermis to methylcholanthrene, not only between the young and old mice of the New Buffalo and CBA strains, but also between both strains.

The amount of lipid was expressed in terms of dry fat-free epidermis. The decrease in the total lipid of the old (12 to 13 months) CBA mice at 17 days after 6 applications of methylcholanthrene was 70% of normal, while the drop in the young mice (3 to 4 months) was nearly 50%. In the New Buffalo group (mice of the same ages) under identical conditions, the diminution of total lipid of the old group was 70% of normal. Other investigations of this laboratory had previously shown that the total lipid:protein nitrogen ratio of young New Buffalo mice fell to 50 to 60% of normal under the same treatment. The depression of total lipid in the old mice of both strains is in agreement, as is the diminution for the young groups of both strains. The findings given above do not help to clarify the age and strain differences with respect to carcinogenic susceptibility, but they are briefly discussed together with the earlier studies with respect to calcium.—Authors' abstract.

The Urinary Partition of Sulphur in Rats Treated with Aromatic Hydrocarbons, with Special Reference to Growth Retardation. Elson, L. A., Goulden, F., and Warren, F. L. [Roy. Cancer Hosp. (Free), London, England] *Biochem. J.*, **39**:301-308. 1945; cf. *ibid.*, **39**:xiv. 1945; abstr. in *Cancer Research*, **5**:730. 1945.

Male rats received a diet containing about 25% of protein and 230 mgm. of sulfur per 100 gm., upon which they grew at the rate of 3 to 4 gm. per day. The amounts of inorganic sulfate, ethereal sulfate, neutral sulfur, and glucuronic acid in the urine were estimated. During a control period each rat received 1 ml. of arachis oil by intraperitoneal injection and subsequently an injection of hydrocarbon in the same volume of oil. Details are given of a special procedure for the estimation of sulfur compounds in rat urine. Benzene caused a rise in the ethereal sulfate without change in the neutral sulfur or glucuronic acid. Naphthalene caused

a large increase in neutral sulfur and raised also the ethereal sulfate and glucuronic acid. Phenanthrene increased the ethereal sulfate, the neutral sulfur, and glucuronic acid. Anthracene caused a moderate increase in neutral sulfur and glucuronic acid. 1,2-Benzanthracene caused a small increase in neutral sulfur and glucuronic acid. 1,2,5,6-Dibenzanthracene (100 mgm. per 100 gm. body weight) given to 10 animals, of which 2 died within 10 days, caused very little change in the excretion. 3,4-Benzpyrene lowered the output of inorganic sulfate but otherwise produced little change. Pyrene caused a considerable increase in ethereal sulfate, neutral sulfur, and glucuronic acid. Chrysene caused no significant change in excretion. The authors point out that their methods would not show very slow rates of excretion. The increased excretion of ethereal sulfate and of glucuronide after naphthalene is probably due to formation of a naphthol, and the very large increase in neutral sulfur is in all probability due to mercapturic acid formation. Boyland and Levi showed that the metabolites of anthracene in the rat included 1,2-dihydroxy-1,2-dihydroanthracene, a glucuronide of this substance, and 1-anthrylmercapturic acid. Phenanthrene is known to yield phenanthryl-glucuronide. It appears that phenanthrol combines with both sulfate and glucuronide, while the hydroxy derivative of anthracene combines more exclusively with glucuronic acid. The more negative results obtained with 1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and chrysene show the difficulty of investigating the metabolism of these compounds.

Three types of effect on growth were observed, namely, (1) the growth rate is unaltered or somewhat retarded (benzene, naphthalene in low doses, anthracene, 1,2-benzanthracene); (2) the body weight falls for several days and then growth is resumed (larger doses of naphthalene, phenanthrene, chrysene, pyrene); (3) growth ceases immediately (3,4-benzpyrene, 1,2,5,6-dibenzanthracene). These data are based upon averages of groups of 10 rats. The authors consider that the data recorded, together with a re-examination of the data of White and White [*J. Biol. Chem.*, **131**:149. 1939], show that the growth retardation produced by such hydrocarbons as 3,4-benzpyrene, methylcholanthrene, and 1,2,5,6-dibenzanthracene cannot be attributed to a need to supply sulfur-containing amino acids for detoxication at the expense of those available for body growth. The results of White and White are criticized. On the other hand, naphthalene causes the excretion of large amounts of neutral sulfur, probably as mercapturic acid, without influencing the rate of growth.—E. L. K.

Nutritional Effects on the Gastric Mucosa of the Rat. I. Lesions of the Antrum. Zucker, T. F., Berg, B. N., and Zucker, L. M. [Columbia Univ., New York, N. Y.] *J. Nutrition,* **30**:301-317. 1945.

Gastric lesions in the mucosa of the antrum of the rat, namely, necrosis, hemorrhage, and epithelial hyperplasia, were found early in calcium deficiency. The lesions were accentuated by phosphate and reduced by vitamin D administration. Deficiency in thiamin or total B complex produced similar small lesions. Dietary calcium supplements abolished the lesions of thiamin deficiency, but added thiamin was devoid of preventive effect in calcium deficiency. Three neutralizing agents used therapeutically, namely, bicarbonates, secondary phosphates, and aluminum hydroxide had no effect on the lesions.—F. L. H.

Nutritional Effects on the Gastric Mucosa of the Rat. II. Lesions of the Fundus and Rumen. Zucker, T. F., Berg, B. N., and Zucker, L. M. [Columbia Univ., New York, N. Y.] *J. Nutrition,* **30**:319-331. 1945.

The authors ascribe fundic lesions (hemorrhages), produced in the rat on deficient diets, to inanition. Hyperplasia and hyperkeratosis of the rat's rumen could be produced by low casein diets in the presence of ample vitamin A and prevented on a totally B-deficient diet by increasing the (purified) casein content to 27%.—F. L. H.

A Study of the Genesis of Histological Changes Produced by Caloric Restriction in Portions of the Endocrine and Reproductive Systems of Strain "A" Female Mice. Huseby, R. A., and Ball, Z. B. [Univ. of Minnesota, Minneapolis, Minn.] *Anat. Rec.,* **92**:135-156. 1945.

Female mice of the A strain were maintained on a diet that restricted caloric intake by one-third for a period of 4 months beginning at the time of weaning. In these animals the ovaries, uteri, and mammary glands were all strikingly underdeveloped and appeared to have been arrested in their development in a prepuberal or juvenile condition. In addition, the juxtamedullary, or X, zone of the adrenal cortex degenerated rapidly after the mice were placed on the restricted diet. This degeneration did not involve the vacuolization that is a characteristic feature of the degeneration that occurs later on in the normal animals.—R. B.

Effect of Adrenalectomy and Ovariectomy on Mammary Carcinogenesis in Strain C3H Mice. Shimkin, M. B., and Wyman, R. S. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **6**:187-189. 1945.

In an attempt to evaluate the relative importance of the ovarian and adrenocortical secretions in mammary carcinogenesis in mice, groups of 4 week old C3H mice were (1) untreated (controls); (2) ovariectomized; (3) ovariectomized and implanted with pellets of desoxycorticosterone acetate; (4) adrenalectomized and implanted with desoxycorticosterone acetate; (5) ovariectomized, adrenalectomized, and implanted with desoxycorticosterone acetate. Whereas 84.5% of the control animals developed mammary cancer, only 33.5, 37.5, 40.0, and 9.3% of groups (2), (3), (4), and (5), respectively, were so afflicted. Hyperplasia of the adrenal cortex was noted in the spayed, nonadrenalectomized mice. Some of the possible hormonal mechanisms involved in reducing the incidence of mammary cancer in the several groups of animals are discussed.—R. A. H.

Relationship between Morphology and X-Ray Effects in Implants of Mouse Sarcoma 180 Irradiated with 5,000 and 60,000 Roentgens (in Air). Goldfeder, A. [New York City Dept. of Hosps., and New York Univ. Med. Coll., New York, N. Y.] *Radiology,* **45**:49-55. 1945.

Fragments of mouse sarcoma 180, irradiated with 5,000 or 60,000 r, showed no increase in size during 10 days following implantation into mice. Extensive degenerative changes were seen in the irradiated tumor cells. No cyto-

logic differences were detected between cells exposed to the smaller dose and those exposed to the larger. However during the last few days of observation, less tumor tissue was detected in the implants of tissue that had received 60,000 r than in the implants of tissue that had received 5,000. There was no evidence that active growth occurred in the tumor implants irradiated with 5,000 r, a dose that had previously been shown to induce resistance.—R. E. S.

A Filtrable Agent Producing Lymphoid Tumors and Osteopetrosis in Chickens. Burmester, B. R., Prickett, C. O., and Belding, T. C. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Cancer Research,* **6**:189-196. 1946.

Manifestations of the filtrable agent or agents in a transplantable lymphoid tumor strain were demonstrated in 3 experiments involving 150 chickens. Inocula containing viable tumor cells induced growth at the site of inoculation, metastasis to the viscera, and death of all birds in a relatively short time (average 10.2 days), whereas centrifuged extracts of the same tumors, or filtered plasma of birds bearing tumors, when injected intramuscularly, intraperitoneally, or intravenously into 2 or 3 day old chicks, induced in 6 months a high incidence of osteopetrosis and lymphomatous tumors of the viscera (average of 81% on gross examination) but no tumors at the site of inoculation. This osteopathy is similar to the avian osteopetrosis, described by other authors, occurring naturally and after inoculation. The lymphomatous tumors in the viscera, particularly in the liver, varied from the diffuse, friable, acute type to the focal, nodular, chronic type. With minor differences these tumors were similar in their gross and microscopic appearance to those observed in other lymphoid tumor strains and cases of visceral lymphomatosis. The results obtained suggest that the avian lymphoid tumor strain under study, which has been transferred serially in over 200 passages by transplantation of its cells, carries with it a filtrable agent or agents capable of inducing osteopetrosis and lymphomatous tumors of the viscera after an incubation period of at least 2 months.—Authors' abstract.

Growth of Trophoblast in the Anterior Chamber of the Eye of the Rabbit. Gurchot, C., and Krebs, E. T., Jr. [San Francisco, Calif.] *Science,* **103**:25. 1946.

Trophoblast from human placentae of about 5-month pregnancies grows rapidly in the anterior chamber and infiltrates the eye.—R. B.

Growth-Promoting and Growth-Inhibiting Factors in Rat Tumor Tissue. Werner, H. [Hebrew Univ., Jerusalem, Palestine] *Proc. Soc. Exper. Biol. & Med.,* **59**:128-129. 1945.

The absence of growth-promoting power in extracts of normal or neoplastic rat tissues may be explained by the assumption that (a) rat tissues are lacking in growth-promoting principles or (b) although present their activity is counteracted by coexisting inhibitors. This report indicates that the absence of growth-promoting factors in rat tumor extracts may be explained on the basis of the second hypothesis. Rat tumor tissue from a benzpyrene-induced sarcoma was finely minced in a Latapie apparatus; the resulting pulp was then extracted with Ringer's solution, and the extract precipitated with 96% alcohol. The precipitate obtained was treated successively for 3 hours in a Soxhlet apparatus with acetone and petroleum ether. After drying *in vacuo,* the extracted powder was suspended in Tyrode solution. The suspension was kept for 3 days in the refrigerator, centrifuged, and the supernatant fluid was added to standardized cultures of chicken fibroblasts in Carrel flasks. The growth of the cell colonies in medium containing this solution as fluid phase was compared with the growth of controls (sister halves) cultured in medium composed of plasma (1 part) diluted with Tyrode solution (2 parts). Culture growth was followed for 6 days. The experimental cultures grew more actively than the controls. The average growth stimulation (13 experiments) was 520%.—M. B.

Studies in Species Adaptation. III. Continuous Exposure of Paramecia to Methylcholanthrene and Other Agents for More than Five Years. Spencer, R. R., and Calnan, D. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **6**:147-154. 1945.

On the basis of previous work with 3 species of bacteria, 2 principles have been evolved: (1) The continuous exposure of actively multiplying, free-living, single-celled, bacterial species to an unfavorable environment may not be fatal to individual organisms or cultures for a number of generations or cell-division cycles, but in due time the strain will die. (2) By discontinuous or alternating exposure, an actively multiplying species can adapt successfully and continue to survive in the same environment that is fatal when the exposure is continuous. The present research indicates that the first of these principles applies also to the protozoan *Paramecium multimicronucleatum* when cultured in media containing a noxious substance. The experiments extended over a 6 year period; the deleterious substances included methylcholanthrene, radium, crystal violet, neutral red, phenanthrene, eosin, and fluorescein. Paramecia that had been exposed to methylcholanthrene continuously for more than 300 days, when transferred to the control environment multiplied much more rapidly than did those that had been cultured in the control media for an equal period of time. The methylcholanthrene-treated organisms retained this "greater survival value" until the 195th transfer in methylcholanthrene or until 10 transfers before the series died out. The problems of cellular and species adaptation are discussed briefly.—R. A. H.

Studies in Species Adaptation. IV. Continuous and Discontinuous Exposure of a Flat Worm to Methylcholanthrene and to Phenanthrene. Calnan, D., and Spencer, R. R. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **6**:155-156. 1945.

Experimentation similar to that carried out on paramecia (see preceding abstract) was extended to the flat worm, *Stenostomum tenuicaudatum,* and evidence was obtained to suggest that the 2 principles of "Species Adaptation" as originally evolved for bacteria were also valid for this species of metazoan.—R. A. H.

Methylcholanthrene and the Environment of Paramecium. Daniel, G. E., Spencer, R. R., and Calnan, D. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **6**:157-160. 1945.

Possible mechanisms responsible for the "greater survival value" previously noted in paramecia exposed con-

tinuously to methylcholanthrene (see second preceding abstract) were investigated. It was found that when normal paramecia, as well as those exposed continuously (MC) or those no longer exposed (MC-lateral), were cultured in a normal medium that previously had been inoculated with the bacterial flora from an MC or MC-lateral culture a much greater population of paramecia was produced than when the media had been inoculated with the flora of a normal culture. If the inoculated media were passed through a filter before the paramecia were transferred to them, the populations obtained were essentially equal. Because of the complexity of the question, no attempt was made to postulate the specific mechanism involved.—R. A. H.

Ocular Tumors with Exophthalmia in Xiphophorin Fishes. Levine, M., and Gordon, M. [Montefiore Hosp., and New York Aquarium, New York Zool. Soc., New York, N. Y.] *Cancer Research*, **6**:197-204. 1946.

Among xiphophorin fishes, *Platypoecilus maculatus, Xyphophorus pygmaeus,* and hybrids between *P. maculatus* and *X. hellerii,* there were sporadic cases of exophthalmia due to melanomas of unknown origin. Examination of these tumors revealed involvement of the choroid, with invasion of the retina and, finally, of the intraocular spaces. These growths were comparable to melanomas of the human eye. Their cells were predominantly epithelioid, but in one instance epithelioid and spindle cells appeared in the same tumor. The cause of the tumors, like that of most animal neoplasms, is unknown. It was not any known microbic or viral agent, nor was it apparently a result of specific hybridizations or controlled by genetic factors. The growths cannot be produced at will, as can generalized integumentary melanomas in platyfish-swordtail hybrids, or certain tumors of plant hybrids.—Authors' summary.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Nervous System

Electroencephalographic Localization and Differentiation of Lesions of Frontal Lobes. Pathologic Confirmation. Yaeger, C. LeV., and Luse, S. [Mayo Clin., Rochester, Minn.] *Arch. Neurol. & Psychiat.*, **54**:197-201. 1945.

This study is based on data concerning 100 consecutive patients, 67 men and 33 women, who had lesions of the frontal lobes of the brain. Surgical intervention showed that in 42 cases the lesion was in the left frontal lobe, and in 47, in the right frontal. The 100 electroencephalographic records were divided into 3 main groups, according to quality of delta wave activity. The pattern of the electroencephalograms in Group I was found to be associated with meningiomas in the majority of cases, and the pattern in Group II was identified with gliomas of all types. The intensity of the changes in the record depended in part on the nature and the extent of the lesion. For the most part metastatic lesions produced electric patterns resembling those of gliomas. Vascular lesions presented patterns similar to those of Group III. In 76 of the cases, delta wave localization gave a correct guide to the position of the lesions.—M. E. H.

The Subependymal Cell Plate (Matrix) and Its Relationship to Brain Tumors of the Ependymal Type. Globus, J. H., and Kuhlenbeck, H. [Mt. Sinai Hosp., New York, N. Y., and Woman's Med. Coll. of Pennsylvania, Philadelphia, Pa.] *J. Neuropath. & Exper. Neurol.*, **3**:1-35. 1944.

A series of 9 neoplasms, the origin of which is traceable to the subependymal cell, is presented. Five of the neoplasms were identified as spongioblastoma ependymale, 1 as papillary ependymoma, and 2 as choroid papilloma. Medullary epithelium, spongioblasts, and neuroblasts are held to be the only clearly recognizable embryonal cellular elements from which all neuroectodermal cell forms arise. There is also found strong evidence for the assumption of the presence of a bipotential mother cell form. Such immature elements are also found within the subependymal cell plate. The structure of this residual periventricular matrix layer, which persists during the entire postnatal life, is surveyed.—A. Cnl.

Primary Sarcomatous Meningioma (Primary Sarcoma of the Brain). Globus, J. H., Levin, S., and Sheps, J. G. [Mt. Sinai Hosp., New York, N. Y.] *J. Neuropath. & Exper. Neurol.*, **3**:311-343. 1944.

A survey of 150 meningiomas subjected to thorough histopathologic study yielded a rather large proportion of sarcomatous meningiomas (16 cases or 9.3%). Of the latter, 8 cases were selected for this study. The histological character and local behavior of these tumors are not unlike those of sarcomas elsewhere. The tumors are frequently multiple, often diffuse, and may give rise to visceral metastases. The cellular constituents of these tumors are commonly traceable to derivatives of the pial component of the leptomeninges.—A. Cnl.

Medulloblastoma in an Adult. Report of a Case. Hare, H. F., and Kassell, M. B. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.*, **4**:145-149. 1945.

The case presented is typical of this brain tumor and its lymphoma-like temporary response to radiotherapy. The unusual localization of symptoms was due in all probability to metastases in the brain and cord.—M. E. H.

Primary Intracranial Chorionepithelioma with Metastases to the Lung. Stowell, R. E., Sachs, E., and Russell, W. O. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *Am. J. Path.*, **21**:787-801. 1945.

Case report.—J. G. K.

Breast

Gynaecomastia in Stilboestrol Workers. Fitzsimons, M. P. [Glaxo Labs., Greenford, England] *Brit. J. Indust. Med.*, **1**:235-237. 1944.

Swelling of the breasts occurred in 20 of 38 men engaged in the manufacture of stilbestrol and in 1 chemist

exposed to dienestrol. Cutaneous and respiratory absorption were believed responsible. Nine case histories are presented, some with biopsy reports, and 1 photomicrograph of mammary tissue is shown.—M. H. P.

Postoperative Prophylaxis of Recurrent Mammary Cancer with Testosterone Propionate. PRUDENTE, A. [Escola Paulista de Medicina, Sao Paulo, Brazil] *Surg., Gynec. & Obst.*, **80**:575-592. 1945.

Sixty-three patients with mammary cancer who had previously been operated upon were treated with relatively large doses of testosterone propionate over prolonged periods. The results in these patients were subjected to detailed analysis as to recurrences and survival during periods of 4 years and more and compared with those in 64 patients with similar growths that were removed in the same way but who were not treated afterwards with testosterone propionate. The analysis led the author to conclude that testosterone propionate exercises a protective or prophylactic action against recurrences of surgically treated mammary cancer. The rationale is given, along with details of the therapy.—J. G. K.

Skeletal Metastases in Cancer of the Breast. BOUCHARD, J. [Roy. Victoria Hosp., and McGill Univ., Montreal, Canada] *Am. J. Roentgenol.*, **54**:156-171. 1945.

In a series of 180 cases of cancer of the breast treated since 1938, there was definite evidence of skeletal metastasis in 37. Of these patients, 24 have died; an analysis of this group is presented. Skeletal lesions were the first metastases to be found and the only ones demonstrable in 62.5% of the cases; these, therefore, as well as pulmonary metastases must be looked for systematically before any operation is performed on the primary tumor, and in regular follow-up examinations. The rate of incidence of bone metastases increases or decreases with the usual rate of incidence of primary cancer of the breast in the various decades. The mean interval between the appearance of the primary tumor and that of the skeletal metastases in the series was about 3 years, except for the age group 20 to 30, in which that interval was but one-half as long. At least 75% of the bone lesions have an osteolytic character when first discovered. An osteoblastic lesion probably indicates a long standing or slowly growing skeletal lesion. In this series, a change from the osteolytic to the osteoblastic type, which is interpreted as the result of depression or death of the neoplasm and repair by the remaining bone, was induced by irradiation. The average survival for the whole group of patients was 13.6 months, the maximum 33 months. Under roentgen therapy, 66.0% of the patients showed subjective improvement; 26.0% showed also objective response to irradiation, the average survival period for the latter category increasing to 18 months, the maximum being 29 months.—E. H. Q.

Osteoid Sarcoma of the Breast: A Complication of Fibroadenoma. ROTTINO, A., and HOWLEY, C. P. [St. Vincent's Hosp., New York, N. Y.] *Arch. Path.*, **40**:44-50. 1945.

Case report, review, and discussion.—J. G. K.

ORAL CAVITY AND UPPER RESPIRATORY TRACT

Carcinoma of the Oral Cavity. A Ten Year Survey in a General Hospital. LAWRENCE, E. A., and BREZINA, P. S. [Yale Univ. Sch. of Med., New Haven, Conn.] *J. A. M. A.*, **128**:1012-1016. 1945.

This is a report of 145 cases of carcinoma of the oral cavity, seen from January 1, 1931, to December 31, 1940; 17 of the cases were in females, 128 in males; only 3 were lost in follow-up. Treatment was usually by radiation, rarely by surgery. The best results were obtained in cases in which the lesions occupied the buccal mucous membranes and anterior tongue; in these cases, 23% of the patients survived 5 or more years. The poorest results were found in the patients presenting hypopharyngeal lesions, 8% of whom survived 5 years. The outstanding cause of failure was inability to control the disease once extension to regional lymph nodes had occurred.—M. E. H.

Carcinoma of the Cheek, Alveolar Processes, Floor of the Mouth, and Palate. BEISWANGER, R. H., and STENSTROM, K. W. [Univ. of Minnesota, and Univ. Hosps., Minneapolis, Minn.] *Radiology*, **44**:213-224. 1945.

A group of 129 cases of carcinoma of the alveolar processes, cheek, floor of the mouth, and palate is analyzed with respect to etiology, metastases, treatment, and end results. The survival rate of 24% compares favorably with the rates reported from other clinics. Treatment was by radiation alone or radiation and surgery combined. The type of treatment for each location is indicated. For better results in the future the increased use of intraoral direct radiation therapy and possibly higher voltage, the more aggressive treatment of cervical metastases, and the earlier recognition of malignant lesions through education of the public, dentist, and physician are recommended.—R. E. S.

Mixed Tumour of Palate. WEBB, S. J. *Brit. Dent. J.*, **77**:320. 1944.

Description of a case of mixed parotid tumor.—E. L. K.

Odontoma Associated with Impacted Cuspid and Retained Deciduous Cuspid. GIERMANN, C. A., and MATLOCK, J. F. *U. S. Nav. M. Bull.*, **44**:827-829. 1945.

A case report.—C. W.

Métastase d'un épithélioma malpighien de la langue dans le ganglion cervical supérieur du sympathique. [Metastasis of a Malpighian Carcinoma of the Tongue in the Superior Cervical Sympathetic Ganglion.] MARTIN, J. F., and DARGENT, M. [Anticancer Center, Lyons, France] *Presse méd.*, **53**:131. 1945.

A case report.—C. A.

Plexiform Neuroma of Tongue. WIGLEY, J. E. M., and MUENDE, I. *Proc. Roy. Soc. Med.*, **38**:505. 1945.

Description of a case.—E. L. K.

A Form of "Mamillated Tongue." PARKES WEBER, F. *Proc. Roy. Soc. Med.*, **38**:334-335. 1945.

Description of a case.—E. L. K.

Carcinoma of the Larynx: Present Concepts of Diagnosis and Treatment. Holinger, P. H. [Univ. of Illinois Coll. of Med., and St. Luke's Hosp., Chicago, Ill.] *Illinois M. J.*, **88**:19-23. 1945.

With a working theory that cancer of the larynx is one of the most curable of all cancers, the author outlines the appropriate curative treatment indicated at various stages of the disease. The care of the cancer is of primary importance; the return of function is of secondary consideration and should not be a determining factor in the choice of therapy. Of 20 patients treated by the author with laryngofissure, only 1 had a recurrence. Of 19 patients with more advanced lesions, subjected to laryngectomy, 12 were living and well at the time of writing, 1 died free of cancer 7 years after the operation, 1 died 3 weeks postoperatively, and 5 had recurrences. These figures are regarded as preliminary, since sufficient time has not elapsed to establish their statistical value.—M. E. H.

Intrathoracic Tumors—Lungs—Pleura

Total Pneumonectomy for Benign Bronchial Adenoma. Tyson, M. D., and Milliken, N. T. [Mary Hitchcock Memorial Hosp., Hanover, N. H.] *Am. J. Surg.*, **67**:111-118. 1945.

Report of a case in which pneumonectomy was decided upon because of the location of the adenoma (too close to the upper lobe bronchus for complete closure of this structure after removal of the lesion) and because of atelectasis of the middle and upper lobes with recurrent infection.—W. A. B.

Isolated Secondary Deposit in a Terminal Phalanx in a Case of Squamous-Cell Carcinoma of the Lung. Smithers, D. W., and Price, L. R. W. [Roy. Cancer Hosp. (Free), London, England] *Brit. J. Radiol.*, **18**:299-300. 1945.

Clinical, radiographic, and pathological photographs illustrate a solitary metastatic deposit in the terminal phalanx of the left little finger in a male aged 49 with bronchial carcinoma. The primary lesion proved histologically to be a squamous cell carcinoma arising by a process of metaplasia from the columnar ciliated bronchial epithelium. The metastatic deposit was of similar histology but nonkeratinized. The bony substance of the terminal phalanx was perceptible only in the form of minute peripheral spicules, and there was incipient malignant infiltration of the soft tissues around the distal extremity of the middle phalanx.—L. W. P.

Malignant Giant Cell Tumor of the Lung. Gnassi, A. M., and Price, P. [Med. Center, Jersey City, N. J.] *Am. J. Roentgenol.*, **53**:582-584. 1945.

Primary sarcoma of the lung is rare. A case is reported with radiographs and photomicrographs. Radiographic examination and postmortem pathological studies ruled out the possibility that the lesion was metastatic.—E. H. Q.

Gastrointestinal Tract

Surgical Management of Carcinoma of the Midthoracic Esophagus. Preliminary Report. Sweet, R. H. [Massachusetts Gen. Hosp., and Palmer Memorial Hosp., Boston, Mass.] *New England J. Med.*, **233**:1-7. 1945.

The relatively new operation involving resection of the entire esophagus below the level of the aortic arch, followed by a high esophagogastric anastomosis either just above or just below the arch, facilitates radical removal of the tumor, including the majority of the regional lymph nodes, and provides a more satisfactory degree of palliation in incurable cases and a better functional result than does the classic Torek operation. A summary of the results of the new procedure in 20 cases is compared with the author's relatively unsatisfactory experience with 14 cases in which the Torek operation was performed.—C. W.

Carcinoma of the Oesophagus. Radical Resection with Oesophagogastrostomy for a Midthoracic Growth by a Right Transpleural Approach. Lewis, I. *Proc. Roy. Soc. Med.*, **38**:483-484. 1945.

Carcinoma of Lower End of Oesophagus. Radical Resection with Oesophagogastrostomy by a Left Transpleural Approach. Lewis, I. *Proc. Roy. Soc. Med.*, **38**:482-483. 1945.

Descriptions of cases.—E. L. K.

Carcinoma Ex Ulcere (?). Editorial. *U. S. Nav. M. Bull.*, **44**:414-415. 1945.

The relationship between gastric carcinoma and ulcer is discussed in the light of U. S. Navy statistics. It is concluded that there is insufficient clinical and pathological evidence to support the concept of carcinomatous transformation of benign ulcer.—C. W.

Gastric Ulcer and Cancer. Allen, A. W. [Massachusetts Gen. Hosp., and Harvard Med. Sch., Boston, Mass.] *Surgery*, **17**:750-754. 1945.

The data for a 10 year period at the Massachusetts General Hospital show that the most helpful factors in arriving at a diagnosis in early malignant lesions are location of the lesion, age of the patient, and duration of symptoms. Nearly all ulcers of the greater curvature and the fundus of the stomach are cancer. In this series, patients over 40 having symptoms of a gastric ulcer for less than 1 year were 5 times as likely to have cancer as benign ulcer. The surgical treatment of any gastric ulcer in this group is recommended. Size of the lesion is misleading in diagnosis. Gastric analysis may be helpful when there is an absence of free acid, as this occurs in at least 60% of patients with cancer. The presence of free acid, however, does not distinguish between the two lesions. —W. A. B.

Longevity with Metastatic Carcinoma of the Stomach. Schwartz, S. O. [Cook Co. Hosp., Chicago, Ill.] *Ann. Int. Med.*, **22**:727-730. 1945.

Metastatic carcinoma was demonstrated in the bone marrow by biopsy 3 years before death.—J. G. K.

Hemangioma of the Intestine. Packard, S. B. [Univ. of Colorado Med. Sch., and Denver Children's Hosp., Denver, Colo.] *Am. J. Surg.*, **67**:556-562. 1945.

A case is presented of hemangioma of the intestine in a 16 year old girl. The patient had a profound anemia and recurrent abdominal pains. A review of the literature is included.—W. A. B.

Lymphosarcoma Primary in the Appendix. Knox, G. [St. Luke's Hosp., New York, N. Y.] *Arch. Surg.*, **50**:288-292. 1945.

Report of a case in a 4 year old boy, in whom the

history of intermittent right lower quadrant pain with nausea and vomiting, and the finding of a palpable mass under McBurney's point, suggested an appendiceal abscess. The lesion proved to be a lymphosarcoma. Twenty-three previously reported cases are reviewed.—W. A. B.

Solitary Giant Follicular Lymphoma of the Vermiform Appendix. Morehead, R. P., and Woodruff, W. E. [Bowman Gray Sch. of Med. of Wake Forest Coll., and North Carolina Baptist Hosp., Winston-Salem, N. C.] *Arch. Path.*, **40**:51-56. 1945.

Report of 3 cases in which a localized lymphoma was found in an appendix that had been removed after the development of symptoms simulating those of acute appendicitis.—J. G. K.

Polyposis of the Colon. Lahey, F. H. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.*, **4**:130-133. 1945.

Early total colectomy in cases of polyposis is needed if the almost certain eventual occurrence of malignancy in the colon or rectum is to be avoided. A case is presented in which 4 carcinomas of the colon arose in a young man as a result of malignant transformation in congenital polyposis.—M. E. H.

Total Colectomy for Polyposis of the Colon with Carcinomatous Degeneration. Wilensky, A. F. [New York, N. Y.] *Surgery*, **17**:630-634. 1945.

Case report. The removed colon showed diffuse polypoid adenomatosis with extensive precancerous changes.—W. A. B.

Carcinoma of the Colon Producing Acute Intestinal Obstruction During Pregnancy. Finn, W. F., and Lord, J. W., Jr. [Cornell Univ. Med. Coll., and New York Hosp., New York, N. Y.] *Surg., Gynec. & Obst.*, **80**:545-548. 1945.

A case report.—J. G. K.

Surgical Treatment of Cancer of the Colon and Rectum. Heyd, C. G. [New York Post-Grad. Med. Sch., New York, N. Y.] *Am. J. Surg.*, **67**:479-487. 1945.

The use of the Devine colostomy as a preliminary procedure for resection of a tumor of the left side of the colon, rectosigmoid, or rectum is described. No deaths followed the procedure in 30 cases.—W. A. B.

The Surgical Treatment of Carcinoma of the Rectum. Statistics on 198 Cases of Resection. Hayden, E. P. [Massachusetts Gen. Hosp., Boston, Mass.] *New England J. Med.*, **233**:81-84. 1945.

The surgical treatment of 198 cases of carcinoma of the rectum in which radical resection was performed is discussed. A 5 year cure rate of 33% was obtained. A single-stage abdominoperineal resection is recommended, the operative mortality in such cases having been 10% compared with an over-all mortality of 13%.—C. W.

Liver

Primary Tumors of the Liver. Warvi, W. N. [Coll. of Med., Univ. of Cincinnati, and Cincinnati Gen. Hosp., Cincinnati, Ohio] *Surg., Gynec. & Obst.*, **80**:643-650. 1945.

A review, and report of experience at Cincinnati General Hospital, with 3 detailed case histories.—J. G. K.

Adenoma of the Liver. Branch, A., Tonning, D. J., and Skinner, G. F. [St. John Gen. Hosp., and Bureau of Labs., New Brunswick, Canada] *Canad. M. A. J.*, **53**:53-54. 1945.

A case report. A classification of these tumors as cholangiomatous, parenchymatous, or mixed is suggested.—M. E. H.

Hemochromatosis Associated with Primary Adenocarcinoma of the Liver. A Case Illustrating Diagnostic Features. Oshlag, J. A., Martin, R. F., and Binford, C. H. [U. S. Marine Hosps., Mobile, Ala., and New Orleans, La.] *Am. J. M. Sc.*, **210**:245-251. 1945.

A case report.—G. J. K.

Bones, Joints, Tendons

Osteogenic Sarcoma of the Skull. Garland, L. H. [U. S. Nav. Hosp., Astoria, Oreg.] *Radiology*, **45**:45-48. 1945.

Osteogenic sarcoma of the skull is a relatively rare tumor, comprising only about 1% of all osteogenic sarcomas. Garland reports a case in a 17 year old male, treated palliatively by surgery and x-ray.—R. E. S.

Synovial Sarcoma. Haagensen, C. D., and Stout, A. P. [Presbyterian Hosp., and Columbia Univ., New York, N. Y.] *Ann. Surg.*, **120**:826-842. 1944.

Synovial sarcomas are composed of two types of cells, those resembling fibrosarcoma cells with accompanying reticulin fibers, and synovial elements that often contain mucicarminophilic droplets and may line slits in the tissue. An analysis of 9 new cases and 95 from the literature is presented, and in only 3 was there survival for 5 years without evidence of metastases. The neoplasm occurred more frequently in males, the average age of appearance was 32 years, and almost half of the tumors were found in the knee. Metastases were disseminated usually by way of the blood vessels but occasionally were found in regional nodes. Although a fatal disease, the average course lasted 5.7 years. Treatment advocated is a limited, incisional biopsy with high amputation immediately after the diagnosis has been made from permanent sections. Dissection of regional nodes may be desirable as a separate procedure. No beneficial effects from irradiation were found.—W. J. B.

Corrections

Volume **5**:602. (Abstracts) 1945. Column 2: line 31-32: for "in the stomach of the host" read "in transplanted stomach tissue"; line 34: for "carcinomas and sarcomas developed" read "carcinomas and sarcomas developed apparently from the transplants." [Editorial errors.]

Volume **5**:729. (Abstracts) 1945. Column 2: line 9 from bottom: for **"47"** read **"Supplement 47."**